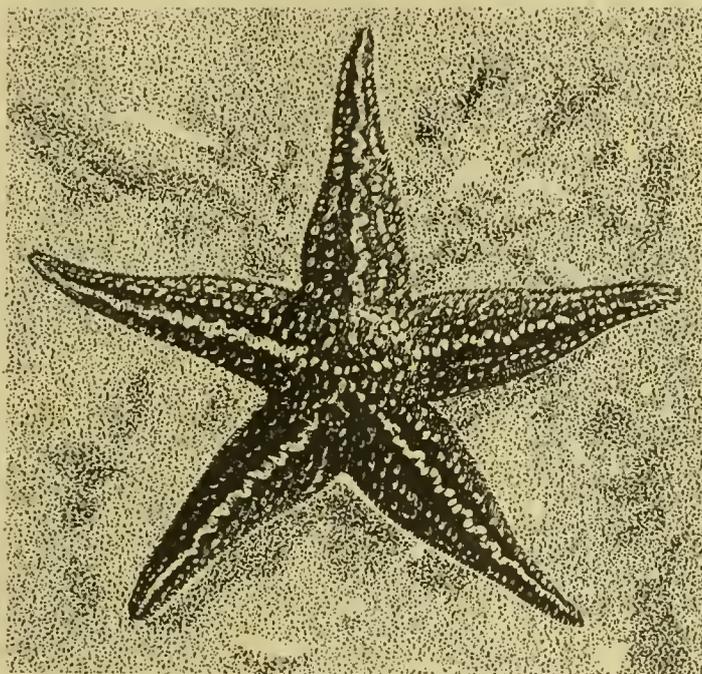


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TECHNOLOGICAL STUDIES OF THE STARFISH



FISHERY LEAFLET 391

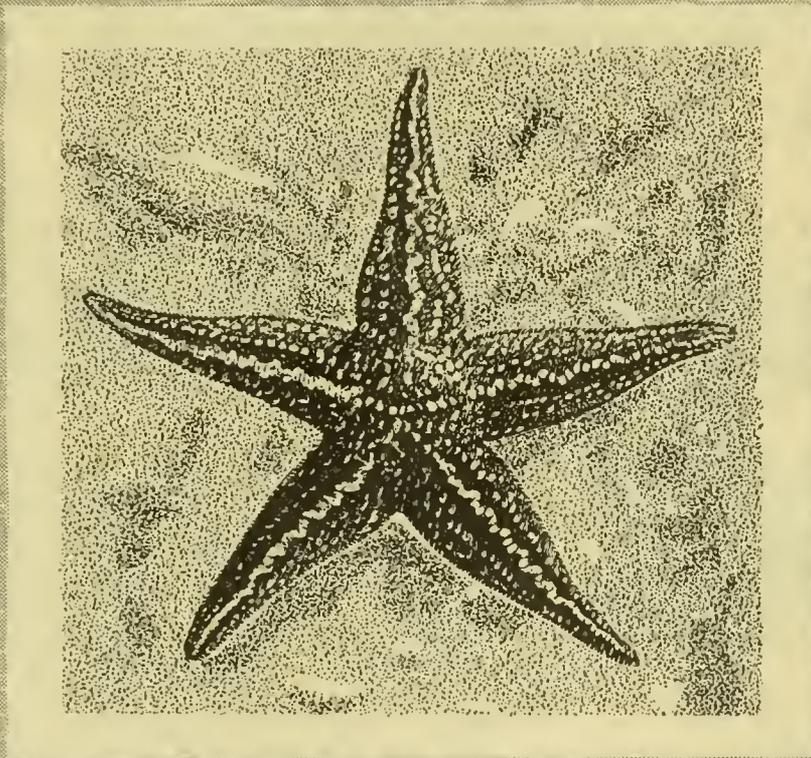
FISH AND WILDLIFE SERVICE

United States Department of the Interior

Washington, D.C.



**TECHNOLOGICAL STUDIES
OF
THE STARFISH**



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Contents

	<u>Page</u>
Part I - Starfish Control--Its Economic Necessity and Methods Used	1
Part II - Chemical Composition	7
Part III - Value of Starfish Meal--Protein Supplement for Growth of Rats and Chicks and for Egg Production	15
Part IV - Thiaminase in Starfish	27
Part V - Starfish as Fertilizer	35
Part VI - Economic Considerations in the Utilization of Starfish	41

Fishery Leaflet 391

Washington 25, D. C.

March 1951

TECHNOLOGICAL STUDIES OF THE STARFISH

PART I--STARFISH CONTROL--ITS ECONOMIC NECESSITY AND METHODS USED

By Charles F. Lee*

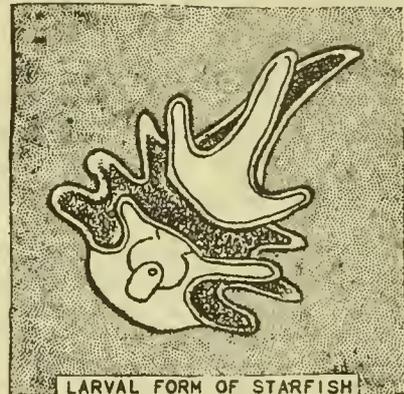
INTRODUCTION

The common five-rayed starfish, *Asterias forbesi*, is a familiar sight in the pools among the rocks of the New England coast. Not so familiar is the fact that the innocent appearing starfish is one of the most destructive enemies of the oyster and that it may cost the oystermen of Long Island Sound over a million dollars per year for control efforts and in seed and market oysters killed.

NATURAL HISTORY AND DISTRIBUTION

In the waters along the shore of Long Island Sound, the lives of the starfish and the oyster are so closely interrelated, that a brief discussion of each is essential to the understanding of starfish control. Galtsoff and Loosanoff (1939) and Loosanoff and Engle (1940) have made extensive investigations of both the starfish and oyster and much of the material presented here represents a summary of information from these sources.

The starfish will spawn when only one year old if conditions for growth have been favorable. Starfish spawning usually starts in June, some two to six weeks earlier than oyster spawning in the same waters. Both the starfish and oyster in the larval form are free-swimming for several weeks before setting on the bottom. When first changed from the larval stage, the young starfish is only about one millimeter in diameter, but it has a voracious appetite and grows rapidly. Having spawned earlier, the young starfish may consume the newly-set oyster spat to the extent of virtually wiping out a good set. For this reason, it is desirable that the beds on which old shells are deposited for the purpose of catching the oyster spat be cleaned of as many adult starfish as possible before they begin to spawn. This will not entirely eliminate starfish, as the larvae in the free-swimming stage may be carried in from some distance by the tide and currents. Such cleansing limits the set, however, and is generally the practice in seed-oyster areas.

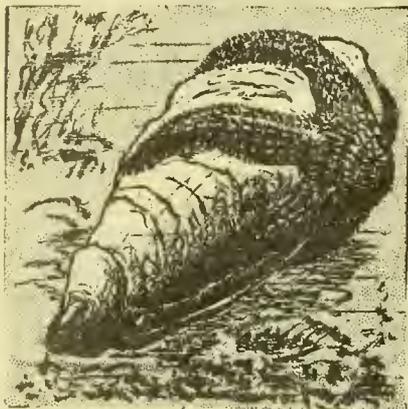


LARVAL FORM OF STARFISH

The oyster industry of the Long Island Sound area is based on intensive private cultivation. In contrast, on the South Atlantic coast, and to some extent in Chesapeake Bay, oysters are taken from public grounds. In the Sound, almost all of the oysters are grown on privately leased beds and, frequently, they may be moved three or more times during the four to six years it takes them to grow to market size.

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The Long Island Sound seed-oyster industry, in many cases, is thus a separate enterprise from the growing of market oysters. Seed-oysters, a term which in this area refers to oysters from one to two years old, are grown almost entirely in a strip of water three to five miles wide along a stretch of the Connecticut coast line from Stamford to Branford, where experience has shown conditions are optimum for obtaining a good set of spat. Even here, for reasons not yet apparent, good sets are obtained only about one year in five, and in some years the set is almost a total failure. To maintain the supply of market oysters at a profitable level, every effort is made to protect the spat and young oysters from unfavorable conditions and predatory enemies, such as the starfish.



The starfish opens the oyster by wrapping its rays about the shell and exerting a steady prolonged pull by means of the many "tube-feet" which line the under side of each ray. These tube-feet are capable of considerable adhesion, but it is the duration, rather than the degree of pull, which gradually fatigues the large muscle of the oyster so that it relaxes and the shell is opened. The starfish then turns its stomach literally "inside

out," to envelop and eat the oyster meat. Some investigators have suggested that the starfish secretes a substance capable of narcotizing the oyster. This ability, if present, is most probably used after the oyster is opened to prevent further closing of the shells.

It is apparent that smaller oysters are more readily and rapidly subject to starfish attack. Therefore, the seed-oyster grower is greatly concerned with starfish control. Large starfish may attack oysters that are three or four years old, but they are more likely to resort to easier prey such as mussels, small clams, crepidula, or several other species of small mollusks.

The surveys of Galtsoff and Loosanoff (1939-40) have shown the depth distribution of starfish to be very similar to that of the oyster of the same waters. Almost all of the cultivated oyster beds, as well as the natural public beds, are in less than 30 feet of water and the great majority of the starfish were found in depths of less than 40 feet. In the wintertime, when the water temperature decreases to 41° F. (5° C.) or lower, the starfish become much less active and many stop feeding. Consequently, destruction of oysters is greatest in the warmer months but control efforts may be carried out the year around.

Although found from Maine to Mexico, the starfish (Asterias forbesi) is rare north of Cape Ann. Although present in southern coastal waters, it is not considered to be a menace to the oyster industry of that section.

The starfish is much more susceptible to changes in salinity than the oyster. This is the controlling factor in Chesapeake Bay and in many sections of the Gulf coast. Starfish do not endure a salinity below 16 to 18 parts per thousand for more than a short time. They, therefore, do not penetrate the Chesapeake Bay much beyond Cape Charles and Norfolk (Loosanoff, 1945). There are a few other high-salinity areas in parts of New Jersey, Virginia, South Carolina, and Louisiana where oysters are grown, but the starfish population is controlled by other factors in these areas. In the open waters of Long Island Sound, however, since the salt content is normally above 25 parts per thousand, salinity is not an important environmental deterrent.

Galtsoff and Loosanoff (1939) made several surveys in different seasons at a large number of stations in Long Island Sound, Buzzards Bay, and Narragansett Bay to study the local geographic distribution of the starfish. Generally speaking, there was no evidence of marked seasonal changes in abundance, within the same year, nor of migration from one area to another. Heaviest concentrations were found where food was abundant, in the western end of the Sound, and in Buzzards Bay near New Bedford and Wareham at the head of the Bay. In Narragansett Bay, near Prudence Island, starfish were plentiful, but relatively few were found in Block Island Sound.

ABUNDANCE OF STARFISH

Starfish have been the subject of control measures by the oystermen of the New England area for most of the 100 years since the beginning of the cultivation of oysters there in 1845.

Among these men, it is common knowledge that starfish on the oyster beds show very large fluctuations in abundance from year to year. Many of these men are of the opinion that decreases in the number of starfish are due to the intensive control efforts that are instituted when it is realized that the numbers are on the increase, and conversely, that the periods of great abundance follow temporary relaxation of control efforts when few starfish are to be found. Migrations from uncultivated areas not subject to control measures are considered largely responsible for maintenance of the starfish population (Anon., 1945).

With the exceptions of a 30-year record by a company on Narragansett Bay and one of 7 years by a company in Connecticut, the oystermen do not have records of how many starfish are eliminated by these control efforts. Their primary interest is in the reduction of the number of starfish to the lowest practicable level. Burkenroad (1946) attempted to determine starfish abundance over a period of some 75 years by a study of trade journals, newspapers, and records of public commissions. Fluctuations in starfish abundance appear to have a definite periodic characteristic, with a range of intervals between the peaks of maximum abundance of 11 to 16 years. This information corroborates the limited data from company records that fluctuations in population are fairly uniform throughout the area involved. Based on Burkenroad's report also, the interesting hypothesis is advanced that the variation in numbers of starfish is due predominantly to natural causes, and is not markedly influenced by the control efforts of the oystermen or by the occasional State or Federal financed efforts toward local elimination. If fluctuation in abundance of the magnitude suggested above were proven, it would require careful consideration whether to recommend utilization, nominal control, or an attempt at complete eradication of starfish.

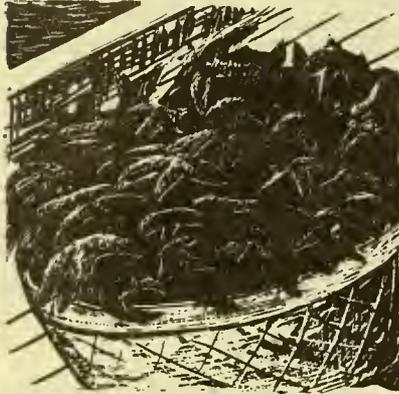
ECONOMIC ASPECTS OF STARFISH CONTROL

An accurate estimate of the damage caused the oyster industry by starfish is difficult to make since it should include not only the direct cost of control efforts, but also the potential value of young oysters killed and the value of marketable seed-stock and older oysters lost. No recent data are available on direct cost of control efforts, but with increased wages and operational costs, it is likely that the total amount spent for this purpose is more than \$500,000 annually.

The oystermen continue these costly controls through the years because they realize what would happen if the starfish were permitted to grow unchecked on the oyster beds.

A single medium sized starfish may kill as many as five one-year-old oysters a day (Anon., 1945). It is possible to calculate the potential loss if a conservative estimate is taken that 100 fair sized oysters are killed a season, and the average weight of a starfish in the Sound is 0.28 pound as estimated by Burkenroad. A bushel of 60 pounds will then contain, roughly, 2,000 starfish. Each bushel destroyed, therefore, represents perhaps 200,000 young oysters that may grow to market size. These would be worth about \$1,000 as one- or two-year-old seed-oysters.

The daily "take" of a vessel engaged in starfishing will vary widely with the type of gear used and the density of starfish on the area worked. Sweet (1946) states that control efforts are carried out even when the amount taken is as low as 10 pounds of starfish per hour per vessel or little more than a bushel per day. On the other hand, in seasons of abundance, the daily average yield may be 25 bushels per vessel per day with maximum yields of 50 to 100 bushels. The usual catch is about 6 to 10 bushels per day on cultivated beds.



Operating costs of a starfishing vessel have mounted rapidly since 1935. A minimum estimate would be \$50 daily when the larger oyster vessels are shifted to these operations. The maximum may be three times this estimate. Depending upon the abundance of starfish, from 5 to 20 or more craft may be used for control purposes. These costly control operations for a non-productive purpose are justified by the potential damage each bushel of starfish is capable of causing if the more than 2,000 starfish it contains are left to continue their depredations throughout the season.

METHODS OF CONTROL

Mopping, dredging, and liming are the methods of starfish control in most general use. Control by other chemical agents; such as, copper and zinc sulfate or chromium salts, has been studied, but none of these methods has proven practical (Galtsoff and Loosanoff, 1939).

Mopping is mostly used both because the mop causes little damage to the delicate seed-oysters and because it effectively and thoroughly cleans areas where few starfish are located. Dredging can be used to clean uncultivated areas free of oysters where the starfish population is very heavy. The regular oyster-dredging operations incidentally capture numerous starfish. These are killed with lime before the oysters are replanted. Liming can be used on either seed or "growing" oyster beds, the chief disadvantage of this method being the difficulty of distributing the lime in proper amounts over the desired areas.

The starfish mop, or tangle, is usually a home-made rig which does not follow any standard design. It is essentially a long bar to which are secured, at regular intervals, 6 to 12 short lengths of chain. Along each chain are tied the "mops," or bunches of string or twine. This outfit is slowly dragged over the bottom at the end of the dredge cable. The starfish become entangled in the mops, are unable to escape, and the mop is hauled up at intervals to remove the starfish.

Starfish may be hand-picked from the mops but the operation is slow and expensive because extra deck-hands are required. Hand-picking may be used on vessels

engaged in abundance-survey operations or on oyster vessels which do not have hot water tanks when pressed into starfish control during emergencies. Most of the seed-oyster companies operate one or more vessels exclusively for starfish control and these are generally equipped with long vats or tanks into which the whole mop frame may be dipped. These tanks are filled with water at a temperature of about 150° F. (66° C.). At this temperature, the starfish are not only killed, but are softened so that they are washed out of the mop as it is lowered for the next dragging operation. Two mops are used, one on each side of the boat, and only about two minutes are required for the hot-water dip. Thus, the mops are in use most of the time and a large area can be covered more effectively than with the dredge or hand-picked mop.

Lime has been found to kill starfish even when only a few small particles settle on the aboral surface. The chemical is only slightly soluble in water and is quite cheap and readily available. The lump lime may be shoveled over the boat rail to be disintegrated and dispersed as it settles to the bottom. Effective coverage in this manner is difficult, as some quantity may be carried away by tide and currents. Loosanoff and Engle (1942) developed an apparatus for distributing a lime suspension immediately over the bottom. A stream of water from a centrifugal pump picked up the fine lime and the suspension was forced through a hose line to a distributor pipe which was carried a short distance above the bottom on a pair of wheels. This apparatus permitseven distribution with little loss to tide and currents, but its use has not been widely adopted because of the expense and difficulty of obtaining the required new equipment.

A fourth control method, the Flower suction dredge, utilized the principle of the vacuum cleaner. A wide funnel-shaped collector was carried on wheels at a short distance above the bottom. The distance could presumably be adjusted to permit removal of either light material only or almost anything loose, including mud and sand. A large centrifugal suction pump discharged this mixture into a rotating screen which separated the larger solid material and dumped it onto a conveyor. It was reported that the desired selectivity of bottom material was hard to obtain and that operating costs were excessively high. Its use would not be justified except in periods of maximum abundance of starfish.

There have been intermittent efforts over a period of years to find some use for starfish, interest in the subject being stimulated by recurring periods of abundance.

The benefits to be derived from the discovery of some economically practical or even profitable means of using starfish would be threefold:

- (1) The oystermen would receive some return for starfish brought in, and inasmuch as all are now discarded, anything received would cut control cost by that extent.
- (2) The creation of a market for starfish would, it may be assumed, lead to independent efforts towards their capture and to new sources of income for certain groups.
- (3) Theoretically, at least, there would be a reduction of the starfish population in the whole area to a point where the peaks of abundance would no longer occur. This event would, of course, simplify the control of starfish on the leased beds and a second, and probably even larger saving to the oystermen would result thereby.

To this end, the Fish and Wildlife Service undertook an investigation of some of the possibilities of starfish utilization. The information obtained in the course of this investigation will be reported in detail in other papers of a series on this subject.

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PART II-CHEMICAL COMPOSITION

By Charles F. Lee*

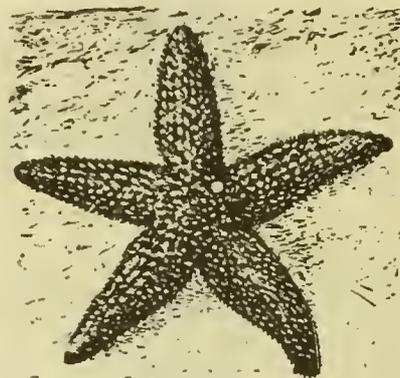
INTRODUCTION

This second section of a series of papers on starfish summarizes all published and available information on the chemical composition of both fresh starfish and starfish meal. The additional information obtained on this subject in the laboratory of the Fish and Wildlife Service is given in detail.

REVIEW OF LITERATURE ON STARFISH COMPOSITION

There are relatively few reports on starfish containing analytical data and those analyses available are, in most cases, not complete. In Table 1, the constituents have been calculated to a uniform basis to facilitate comparison. For example, calcium or calcium oxide are reported as calcium carbonate.

The data are seen to be rather fragmentary, but sufficient to show that starfish are not of constant composition with regard to any single constituent, even when values are calculated on a dry matter basis. Protein, ether extract (fat), and ash with its chief constituent (calcium carbonate), all show a large degree of variation. The data of Hutchinson, et al., (1946) are from an analysis of a small laboratory sample dried at 57° C. (134.6° F.). These values are referred to in several other papers as the composition of the sun-dried meal which was supplied by the Bingham Oceanographic Laboratory group to other laboratories for cooperative studies. That this inference was entirely justifiable is doubtful, as indicated by the check analysis of this same meal reported by Whitson and Titus (1946). Loss of about 10 percent of the protein originally present in the fresh starfish apparently occurred during preparation of the sun-dried meal.



The data of Morse, et al., (1944), also reported in this group of papers, were obtained on a commercially dried experimental batch of four tons of starfish, although the results of their laboratory sample analysis is comparable to data of Hutchinson, et al. A difference equal to 22 percent more protein and 30 percent less ash in the laboratory meal than in the commercial sample is indicated by comparison of these data.

Vachon (1920) emphasizes even more clearly the probable difference between a commercial starfish meal, and a specially prepared sample in the two analyses he reports, one of the material as collected, with seaweed, shells, sand, and other adhering matter as would be used in the practical preparation of large quantities of starfish meal, and the other, the analysis of starfish washed several times and separated from all foreign matter. The value reported for protein in this latter analysis appears to be erroneous.

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NOTE: Part I of this series appeared in the January 1948 issue of Commercial Fisheries Review, pp. 1-6. Also Separate No. 193.

Table 1 - Data in the Literature on the Composition of Starfish and Starfish Meal

Type of Sample and Reference	Dry Matter Percent	Protein (Nx6.25) Percent	Ether Extract Percent	Crude Fiber Percent	Calcium (asCaCO ₃) Percent	Magnesium (asMgO) Percent	Phosphorus (asP ₂ O ₅) Percent	Potassium (asK ₂ O) Percent	Sulfur (asSO ₄) Percent	Sand (SiO ₂) Percent	Total Ash Percent
Fresh Starfish: Wheeler (1913)	25.12										10.9
Galtsoff, et al (1939)											
Hutchinson, et al (1946)					14.0		0.34		0.87		
Laboratory Dried and Specially Cleaned Meal: Kole, C.J. (1919)	87.9	31.6	6.9							3.9	43.9
Vachon, A. (1920)		55.7								0.57	
Hinard & Fillon (1921)		35.62	7.53		47.7		1.3	2.42	1.65		52.95
Morse, et al (1944)	96.89	33.63	7.7	0.4	37.9		1.1				41.7
Hutchinson, et al (1946) ^{1/}		34.0			42.0		1.0	2.61			
Commercial Type Meal: Vachon, A. (1920)		11.0									
Galtsoff, et al (1939)	89.9	36.3		1.84	69.8	3.25	0.70		0.73	3.46	
Morse, et al (1944)	47.9	27.54	5.3								60.13
Whitson & Titus (1946)	94.5	30.7	4.5	1.9	44.0		0.80				30.0 ^{2/}

^{1/}Also Iron - 0.017%, Manganese - 0.0023%, Fluorine - 0.016%, Boron - 0.0034%, Thiamine - 0.0001%, Riboflavin - 0.00053%, Niacin - 0.0038%, and Pantothenic acid - 0.0012%.

^{2/}Ash was ignited at 900°C. - CaCO₃ loses CO₂ at 825°C. so figure for CaCO₃ is calculated from value for Ca.

Noddack and Noddack (1939) made an extensive spectrographic study of the various metallic elements in a number of marine animals, including starfish. In general, they found greater concentrations of the heavy metals in the animals than in sea water, but quantities were still on the order of one part per billion, with starfish showing the smallest total concentration. Boron was the only element notable in starfish, with 1 to 10 parts per million.

The purified fat of starfish has been reported by Hinard and Fillon (1921) to have a density of 0.9372, an iodine number of 132.7, a saponification number of 159.1, and unsaponifiable matter content of 38.94 percent.

In general, these data from the literature indicate that composition of commercial meals cannot be predicted from analyses of fresh samples of raw starfish. There is, apparently, a loss of about one-tenth or more of the nitrogen present. This could result from a rapid breakdown to soluble products of a portion of the proteins present and their loss in body fluids. Ash is likely to be considerably higher in commercial meals than in laboratory dried meals. This results, in part, from the inclusion of oyster shell, and many other small shellfish, as well as sand and small rocks that are taken in the dredge along with starfish. This variability in analyses is quite evident in data obtained in the present investigation.

MATERIAL

The lack of drying facilities near the source of the starfish at Milford, Conn., made it necessary to ship the starfish, while fresh and perishable, to the College Park Laboratory, some 300 miles distant. As a result of this, the analyses of the fresh starfish cannot be considered representative of the live starfish immediately after catching. However, the data for the meals are a good approximation of what might be expected of commercial meals receiving ordinary care in transportation and handling.

Starfish were obtained at Milford, Conn., by dredging or were hand-picked from mops through the cooperation of a commercial company and the Fish and Wildlife Service Biological Laboratory at Milford. Lots of 50 to 100 pounds were shipped, either in 5-gallon oyster cans or in small, tight, wooden kegs. Samples in the cans were lightly iced, while cool weather alone limited decomposition of the other lots. On arrival at the laboratory, a considerable volume of free liquor was found to have separated in every case. In some lots, the starfish had undergone some decomposition, though most were in good condition, bright colored, and hard.

PREPARATION OF SAMPLES AND ANALYTICAL PROCEDURES

Portions of the liquor on several of the earlier lots were tested for total solids, salt, ash, and organic material. These data are reported in Table 2. The

Table 2 - Proximate Analysis of Liquor Separating from Starfish

Source	Total Dry Matter	Ash	NaCl ^{1/}	Organic Matter	Source	Total Dry Matter	Ash	NaCl ^{1/}	Organic Matter
	Percent	Percent	Percent	Percent		Percent	Percent	Percent	Percent
Lot 1 A	4.63	2.77	2.11	1.86	Lot 2	2.02	1.26	0.95	0.76
" 1 B	4.67	2.63	1.98	2.04	" 3	3.95	2.88	2.42	1.07

^{1/}Total chlorine calculated to sodium chloride.

content of total solids was found to be about 4 percent, of which about 60 percent was inorganic (ash). Sodium chloride calculated from chlorine content constituted approximately 75 percent of the ash. Because of the inadequacy of the available

drying equipment and the small amount of organic matter present in this liquor, it was not considered desirable to add it back to the starfish, so the liquor was discarded. The small amount of nitrogen thus lost would not have been retained in usual plant handling practice.

The bulk of the starfish for meal was dried in large galvanized pans in steam heated ovens. The starfish were several inches deep and matted down to a dense layer which retarded drying and the maximum temperature obtained in the oven was 60° C. (140° F.). As a result, complete drying required 5 to 7 days, even when the matted layer was stirred and broken up daily.

In the initial stages of the drying operation, temperatures were sufficiently low to encourage vigorous enzymatic and probably bacterial action. This was desirable since it resulted in a break-down of the exoskeleton of the starfish and greatly facilitated the grinding of the dry meal. It was virtually impossible to grind the tough and hard structure resulting from rapid drying by any means available. Generally, the decomposition was stopped by further drying before it had reached the stage of liberation of ammonia and darkening of the meal, though this did occur in one or two large batches.

METHODS OF ANALYSIS

Dry matter was determined by heating overnight in an air oven, at 105° C. (221° F.). Ash was obtained by ignition of the dry material at 600° C. (1112° F.), until grayish white, and chlorides were determined on the ash by leaching with 1 nitric acid to 3 water and titrating a suitable aliquot by Volhard's method. Total nitrogen (N x 6.25 to give crude protein) was determined by Kjeldahl digestion using copper sulfate as a catalyst. Total organic matter was calculated by difference.



The solvent-soluble portion of the starfish was extractable with ether, but was more readily soluble in acetone. Most of the data on this constituent were obtained from bulk extractions with a Soxhlet type extractor using a mixture of acetone and petroleum ether. The solvent was recovered by distillation and the last portion was removed with aid of a vacuum. Considerable quantities of starfish oil were thus prepared for an investigation of the sterols present in starfish by Dr. Werner Bergmann

and coworkers of the chemistry department of Yale University.

In view of the toughness of the starfish "skin," it was thought likely that a chitin-like material might constitute a major portion of the protein present. Chitin was therefore determined on two samples by digestion of the fat-free starfish meal with 20 percent KOH at 60° C. (140° F.) for two weeks. Chitin was determined as the loss on ignition of the dried residue at 550° C. (1022° F.) since the carbonate ash constituted the bulk of the undigested material.

ANALYTICAL RESULTS

The proximate analyses of the meals and some lots of fresh starfish are tabulated in Table 3. Data are not complete on all meals, as several were prepared for special purposes, such as for feeding tests or for the extraction of oil.

All the data for solvent extract except the first value were obtained from bulk extractions, in which the ground meal was placed in canvas bags in the extractor. Caking of the large bulk of meal prevented complete extraction of oil so that these values are perhaps 5 to 10 percent low.

Table 3 - Proximate Analysis of Some Samples of Fresh Starfish and Starfish Meal

Lot	Dry Matter	Protein	Solvent Extract	Total Organic Matter	Chlorides as NaCl	Total Ash
	Percent	Percent	Percent	Percent	Percent	Percent
Fresh Starfish:						
1	35.2		2.7	15.8	2.18	19.4
2	35.0			14.1	1.47	20.9
4	33.6			16.5	1.24	17.1
5	32.6			16.6	1.10	16.0
7	36.0			14.7	1.03	21.3
Starfish Meal:						
1	99.2		7.6	44.4		54.8
2	97.2	27.9	6.9	40.9	2.85	56.3
3	98.7	26.3		39.3	3.81	59.4
4	97.9	29.9	15.1	50.5	3.29	47.4
5	99.1		6.8	42.2	3.73	56.9
6	99.0		8.7	40.1	3.90	58.9
7	99.4		9.3	38.5	3.14	60.8
8	94.9		9.0	36.4		58.5
9	97.2			39.3		57.9
10		28.1				
11		30.6				

Generally, the solvent-soluble material constituted about 7 to 9 percent of the meal. The one high value for Lot 4 of 15.1 percent was obtained with starfish which were full of spawn. As most of the samples were collected in October and November, normally they were spawned out, and this fact may, in part, account for the lower values for protein, as well as oil, and higher ash content than have usually been observed for this species. This particular meal from Lot 4 was the only one to have an ash content under 50 percent. The others ranged from 55 to 61 percent ash, in spite of the fact that an effort was made to pick out most of the shell and foreign matter before drying.

The protein content, as noted before, is lower than some values previously reported, five meals averaging 28.6 percent. Seasonal differences, loss in liquor, and loss during drying may all have contributed to make these low values. The data did not cover a long enough period of time to permit evaluation of all the factors involved in the variable composition of the starfish.

It is evident from the data that the meal dries readily even at the low temperatures used, several meals containing less than one percent water. The starfish before drying contained about 35 percent dry matter, compared to the value of 25 percent reported usually used as a meal factor for freshly caught starfish, indicating a loss of about 10 percent of liquor which separated in transit. Salt content of the meals is well within the permissible limits for fish meals. Chitin content, representing indigestible protein, was found to be low, only 0.55 percent of the dry meal.

The unsaponifiable portion of starfish oil has been examined, particularly in regard to the sterols present, by Bergmann and coworkers. In a preliminary report, Bergmann (1937) had reported that the sterol of starfish, named stellasterol by Kossel and Edlbacher (1915) was a mixture of two or more sterols which were extremely difficult to separate. This sterol mixture, as well as the alcohol, astrol, were present in Asterias forbesi.

This work had been halted by lack of sufficient material until 1942 when the College Park Laboratory supplied Dr. Bergmann with about 12 pounds of oil to permit further investigation.

In 1943, Bergmann and Stansbury reported that the alcohol in starfish oil called astrol by Kossel was identical with batyl alcohol, previously observed in liver oils of sharks and rays. Batyl alcohol is glycerol - 1-octadecyl ether. The structural formula is thus: $\text{CH}_3(\text{CH}_2)_{17}\text{O}\cdot\text{CH}_2\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$.

In 1944, in a second report on the sterols of starfish, Bergmann and Stansbury had not yet succeeded in complete separation of the sterols. Much had been learned of their molecular structure, however, by selective hydrogenation of the mixture and by other means. The two sterols, tentatively called stellasterol and stellastenol, apparently differed only in the presence of two double bonds in the former, while stellastenol had only a single double bond. Both were slightly dextro-rotatory in contrast with all other animal sterols, therefore, they must lack C 5-6 double bond linkage, having instead a double bond at the Carbon 8. Some of the difficulties in separation and purification of the sterols are thought to be due to a shifting of this double bond from the gamma or delta to the alpha isomer.

These sterols are the first of the principal unsaturated sterols of 28 carbon atoms to be reported in animal tissues. Saturated stellastenol was apparently isomeric with campestanol.

In a more recent publication, Bergmann, *et al*, (1945) suggest that recent observations support a hypothesis that stellasterol when hydrogenated yields a mixture of campestanol and a 24-carbon atom isomer, and that the starfish sterols are mono- and di-unsaturated derivatives of campestanol, the second double bond being in the C 22-23 position. Some sponge sterols are also related to this compound.

The general study of the sterols of marine invertebrates, contrasting in its complexity with the simple sterol make-up of land animals, is of considerable theoretical interest, especially in connection with theories of the origin of sterols in the body tissues.

NITROGENOUS CONSTITUENTS

The present investigation did not include a study of the amino acids present in starfish proteins. The separation and identification of amino acids was a problem of too great complexity to approach with the personnel and the methods available. In recent years, however, there has been a rising interest in amino acids as special dietary supplements, in medicine, and in nutrition research.

Kossel and Edlbacher (1915) made the only study of amino acids in starfish reported in the literature, and they found taurine in the free state in the sexual organs. Glycine, tyrosine, and glutamic acid were isolated from another fraction of the hydrolysate, while sarcosine, leucine, isoleucine, and proline were also identified. The monoacids, not precipitated as phosphotungstates, accounted for 39 percent of the total nitrogen of the dibasic amino acids. An arginine content of 19.4 percent and lysine content of 11.5 percent were most prominent. Also noted was a small amount of a "histidine fraction." This work was part of an extensive chemical study of starfish, and does not represent any attempt to work out practicable methods of separation. The possibilities for preparation of the various amino acids from starfish, as well as other marine products or byproducts remains virtually unexplored.

It is quite probable that this phase of the utilization of starfish will be emphasized in further investigation of starfish now being considered. The study may be simplified by use of the recently developed microbiological methods for the assay of many of the amino acids, and may result in development of a much more profitable outlet for a product from starfish than the meal and fertilizer preparations hitherto studied.

CONCLUSIONS

The data on the composition of starfish show that the fresh material will yield about one ton of meal per four tons of raw material. The commercial meal contains about one-half as much protein as the common commercial fish meals, but compares favorably in this respect with meals prepared from crab or lobster scrap, shrimp bran, or meals from mussels or other of the less desirable shellfish.

The meal is high in calcium carbonate, while potassium and phosphorus are disproportionately low. Starfish oil contains batyl alcohol, and two newly discovered sterols with 28 carbon atoms related to campesterol. The protein of starfish contains some of the essential amino acids and appears to be a more probable source of a valuable extractive than is the oil fraction.

The use of starfish meal as a protein supplement in rat and chick growth tests and in laying mashers will be reported in a third paper of a series on the utilization of the starfish, Asterias forbesi.

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PART III-VALUE OF STARFISH MEAL--PROTEIN SUPPLEMENT FOR GROWTH
OF RATS AND CHICKS AND FOR EGG PRODUCTION

By Charles F. Lee*

INTRODUCTION

This is the third paper in a series of six technological studies of the starfish (*Asterias forbesi*). The first discussed its ecological relation to the oyster in Long Island Sound, the necessity for starfish control by the oyster industry, and the control methods used. The second paper reviewed data on chemical composition of the starfish. The work of Dr. W. Bergmann on the sterols of starfish was also discussed briefly. The present paper is concerned with the utilization of starfish meal in starting and laying mash for poultry and in diets for growing rats.

REVIEW OF LITERATURE

Although efforts to exterminate starfish have been carried out by oystermen on Long Island Sound for nearly 100 years, almost no effort has been made towards the utilization of starfish so taken in these control efforts.

After the first World War, in 1919, K le reported the utilization in Germany of starfish for feed as well as for fertilizer, but it seems to have been used chiefly to adulterate the more valuable shrimp meal. Vachon (1920) suggested that starfish might be used as a fertilizer in Canada if a sufficient supply of raw material were available, and Gibbs (1941) reported that the raw starfish which were brought in for payment of bounty in Rhode Island in 1941 were used locally by farmers and by State institutions as fertilizer.



However, it remained for the period of World War II, with its accompanying shortage of protein feeds, to cause an extensive investigation of the possible use of starfish meal in feed mixtures. In recent years, the use of commercially mixed poultry mash and other feedstuffs has increased rapidly. Fish meals

have proven of exceptional value as sources of proteins of a type not found in any vegetable source and the established fish meal industries using menhaden, herring, pilchard, and the so-called "whitefish" fillet scrap have been unable

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NOTE: Part I of this series "Starfish Control - Its Economic Necessity and Methods Used," appeared in the January 1948 issue of Commercial Fisheries Review, pp. 1-6. Also available as Sep. No. 193.

Part II, "Chemical Composition," appeared in the February 1948 issue, pp. 11-18. Also available as Sep. No. 196.

to keep pace with the increased demand. All of these factors have accelerated the search for other sources of marine, high-protein meals for use in feeds.

There have been 6 papers published since 1944 by different groups of investigators dealing with the use of starfish meal as a protein supplement for the feeding of newly-hatched chicks. All but one of these reports presented results of work instigated by a group at the Bingham Oceanographic Laboratory which has been interested in the development of unutilized marine resources of southern New England. These reports will be briefly summarized. Bird (1944) fed poultry 3 and 6 percent starfish meal in a basal mash adjusted to maintain so far as possible the calcium:phosphorus ratio and protein content at the same levels as the control diet which contained 4 percent of fish meal. Growth was substantially equal with all three diets. The differences of 3 and 4 percent, respectively, lower mean gain in liveweight of the groups fed 3 and 6 percent starfish meal were not significant. Shank color was not bleached when the starfish meal was fed.

Heuser and McGinnis (1946) also fed to chicks diets containing 3, 6, and 12 percent starfish meal. Growth with the former diet was equal to that of the control group fed a diet containing 3 percent fish meal. There was about a 7 percent decrease in the mean gains of liveweight of the group fed the 6 percent level as compared with the control group. Chicks receiving a 12 percent level of starfish meal showed 16 percent mortality and significantly poorer growth. The gain in liveweight was only 61 percent of that of the control group. The diets contained equal quantities of protein so it was concluded that the excess calcium was responsible for the poor results obtained at the 12 percent level of starfish meal in the diet. It was concluded that the 6 percent level was the largest amount that could be fed with reasonable success.

Ringrose (1946) compared diets containing 13 and 18.5 percent levels of crude protein when fed to chicks. Mashers with the 13 percent protein content contained either 9 percent starfish meal, 4 percent rosefish meal, or 5 percent meat scrap, respectively, while those with 18.5 percent protein contained double these quantities and each diet also included 10 percent soybean oilmeal.

Growth was poor with all diets at the 13 percent level of crude protein. The diets containing starfish produced 83 percent of the gain in liveweight of that produced by the diet containing rosefish meal. With the high-protein diets, the chicks fed the 18 percent level of starfish meal averaged only one-third the gain in liveweight of the control group, and also showed a 50 percent mortality. Again the poor results are attributed by the author to the large calcium:phosphorus ratio, the high calcium content, or both.

Stuart and Hart (1946) fed chicks a diet containing starfish meal at a level of 4 percent supplemented with 4 percent meat scrap and 4.5 percent fish meal. The control group received a diet containing 7 percent fish meal and 4 percent meat scrap. Over a 12-week test period, rates of growth with the two mashers were approximately equal. Analyses of the tibia showed a higher calcium and ash content in the bones of the group which had received starfish meal than in those of the control group.

Whitson and Titus (1946) fed 3 series of chicks to make a more critical study of the quality of the protein of starfish meal. In the first series, 4 and 8 percent; in the second series, 4, 8, and 12 percent; and in the third series, 2.5 and 7.5 percent of starfish meal were included in the diets. In every case, starfish meal was the sole source of animal protein. Sardine meal was used similarly

in the various control mashes, and varying amounts of ground limestone, soybean oilmeal, and wheat were replaced by these fish meals to balance the nutrients. Growth rates of the chicks fed mashes containing the lower levels of starfish meal were as good as, or better than, those obtained with the control mashes, but the rates were lower for the groups fed the 8 and 12 percent levels of starfish meal. The mean gain in liveweight of the group fed the 12 percent level of starfish meal was only 60 percent that of the control group. It was concluded that the starfish meal could be used to supply all of the calcium and some of the animal protein, it having the same growth-stimulating qualities as sardine meal protein. The calcium content limited the amount of starfish meal which could be used.

Morse, *et al*, (1944) carried out their study with starfish meal with an experimental lot of about one ton of meal produced in commercial scale operations. The starfish meal was included in diets at 4 and 8 percent levels and compared with those containing a 4 percent level of crab meal and a 2.5 percent level of fish meal. The protein level and calcium:phosphorus ratio were approximately balanced. There were no significant differences in mean gains of liveweight between any of the four groups of chicks after 8 weeks. Smaller groups were continued on experiment and fed the diets containing 2.5 percent fish meal and 8 percent starfish meal until the 14th week. At this time, the two groups were still about equal in size and feathering. At this level, the starfish meal plus dicalcium phosphate adequately replaced both the fish meal and meat scrap.

Although the tests varied somewhat in detail of experiment, all of these investigators have used newly-hatched chicks. Their conclusions agree in substance; namely, that small amounts of starfish meal can replace other animal proteins as a source of supplementary protein permitting approximately equal growth on an equal-protein basis. When several levels were fed, poorer growth usually resulted when more than 6 percent starfish meal was included in the diets. It was generally concluded that this effect was due to the resultant high levels of calcium, to the unbalanced calcium:phosphorus ratio, or to both factors.

STARFISH MEAL FOR GROWTH OF RATS

The work reported herein antedates the 6 papers just discussed, having been carried out in the summer and fall of 1942. This fact is mentioned in explanation of the inclusion in these tests of certain preliminary studies exploring the possible effects of the high levels of calcium in starfish meals.

The method of preparation of the meals used in these feeding tests has been described in Part II of this series dealing with chemical composition. In brief, starfish drained of free liquid which had separated in shipping were dried in ovens heated by steam coils at about a temperature of 60° C. Most of the foreign matter was removed before drying. In the first rat and chick tests, the extracted meal referred to was that remaining after the starfish oil was extracted. A quantity of this meal was available and it was fed in amounts equivalent to the protein in the diet containing the highest level of starfish meal fed to find out whether the oil had an adverse dietary effect which had to be considered in producing feeding meals.

As a preliminary to the feeding tests, the nutritive quality of the protein was determined by a nitrogen metabolism study. Six adult male rats were fed a protein-free diet during a preliminary and following period of 10 days, and the starfish protein to be tested was then fed during a middle 4-day period according to the method of Mitchell (1924). Feces and urine were collected for each period.

A determination of the nitrogen excreted at known intake levels permitted calculation of the digestibility and biological value of the protein fed. The average value for digestibility was 76.4 percent, while the average biological value, indicative of the availability of the protein, was 83.9 percent. These values compare favorably with similar data on other types of fish meals.

In the first series of feeding tests, rats and chicks were started at the same time with similar diets. The composition of these diets is given in Table 1 (see page 12). Corn, wheat middlings, and pilchard meal levels were varied with the starfish meal so as to equalize the calculated crude protein content in all diets. The nitrogen content was later determined by the standard Kjeldahl method, the results of which agreed with the calculated values quite closely. No attempt was made in either of the test series to compensate for the high level of calcium and the highly unbalanced calcium:phosphorus ratio, since it was desired to determine the extent of the tolerance for the calcium of the starfish meal which was unavoidably included along with the more sought-after protein. The calcium and phosphorus content of the diets has been calculated (Table 1) and the calcium:phosphorus ratios for the highest levels of starfish meal fed were found to be about 24 to 1 for the diets fed to rats, and 11 to 1 for those fed to chicks.

Ten rats weighing 48 to 55 grams each, evenly divided as to sex, were used in each of the 5 groups. The tests continued for 6 weeks, and the liveweight and feed consumption of each rat were recorded weekly. The results showed that the groups receiving 12 and 24 percent starfish meal had a lower growth rate than the control group while those receiving 48 percent starfish meal and 43 percent extracted starfish meal showed a net loss of from 8 to 12 grams from the initial weight. The surprising fact is that only 4 of the 20 rats in these 2 groups died. These deaths did not occur until the 6th week, and the remaining rats, while extremely emaciated, were quite lively and showed no other gross symptoms of damage. There were no deaths for the groups fed the 12 and 24 percent levels of starfish meal, although mean gain in liveweight in these groups was only 58 and 27 percent, respectively, of that of the control group.

Early in the test, it was observed that the rats tended to sort out and leave the starfish meal. This was corrected after the second week by grinding all meals very finely in a ball mill. It is interesting to note that, at this degree of fineness, the meal was so hygroscopic that the diets caked in the feed cups. The constituent responsible for this property is not known. The caking did not seem to affect the feed consumption, which remained at a fairly even level throughout the test period.

Feces were collected from 3 rats in each group during the last 3 days on tests and analyzed for total nitrogen. The mean values for the apparent digestibility of the protein in the diet indicated by these analyses were as follows: for the control group, 86.3 percent; 12 percent starfish meal level, 80.6 percent; 24 percent starfish meal level, 80.9 percent; 48 percent starfish meal level, 79.2 percent; and for the rats fed the high level of extracted meal, 81.7 percent. This high and relatively uniform degree of digestibility at all levels would seem to indicate that the poor growth was not a result of interference with protein metabolism.

Presence of thiaminase, the thiamine destroying enzyme, had previously been demonstrated in fresh starfish, and the results of the chick growth tests indicated that some of this substance still remained in the meal. However, there was little or no improvement in either weight or condition of the rats with the addition of

one microgram of thiamine per gram of diet. In this series, the poor growth was apparently not due primarily to a thiamine deficiency.

The carbohydrate contents of the diets containing starfish meal were lower than that of the control diet but hardly to an extent which would account for the extremely large differences observed. In fact, the only explanation for the poor results of feeding starfish meal to rats appears to be that the rat will not tolerate the serious imbalance of the calcium:phosphorus ratio which resulted from the large excess of acid-soluble calcium contained in the starfish meal.

STARFISH MEAL FOR GROWTH OF CHICKS

It was evident from the tests with rats that this animal is not satisfactory for assaying diets containing high levels of calcium. Results obtained from a series of feeding tests with chicks were much more satisfactory and have the further advantage of being directly applicable to commercial practice. The poultry feed industry is probably the largest single user of high protein meals. Chicks and hens were therefore used in all subsequent feeding tests.

All chicks were a Rhode Island Red-New Hampshire cross, purchased from a nearby hatchery. Chicks were housed by groups in batteries in a room in which the temperature was maintained at 78°-82° F. Birds were distributed between the groups at random, although an effort was made to have initial weights of the groups about equal. Size of groups was limited to 10 chicks each by the small size and limited number of batteries available. Birds were weighed individually at weekly intervals and group feed-consumption records were kept. These data are not highly significant, since there was some wastage in scattered feed.

The same starfish meal was used for the first series of rat and chick tests and similar levels were fed. Bran, soybean oilmeal, and dried skim milk supplied additional phosphorus to reduce the calcium:phosphorus ratios materially below those of the diets containing comparable levels of starfish meal which were fed to the rats. The composition of the mashes is presented in Table 1. The crude protein was maintained at a 23.5 percent level, primarily by the adjustment in the amount of pilchard meal. Starfish meal was fed at 8, 16, and 32 percent levels. These are really abnormally high levels and were fed with the sole intention of determining the tolerance of the chick for excessive calcium content with no compensating sources of phosphorus. The amounts of calcium and phosphorus, calculated from tables of feed analyses and the calcium:phosphorus ratios are presented in this table also.

A second series of chicks was fed in the same manner as the first, with diets modified on the basis of the results obtained in the first test. In this second series, starfish meal was fed at 3, 6, and 9 percent levels in order to determine the level which permitted optimum growth. The composition of these diets is given in Table 2 (see page 12).

In the first series with chicks, the control group made rather poor growth, for reasons to be explained later. The group fed the lowest level of starfish meal is used as a basis of comparison in this case. It was difficult also to find a basis of comparison by which the two groups fed the highest levels could be included, because only one-half of these groups survived, and these would have died except for supplementary thiamine supplied after 3 weeks. These chicks made small gains in weight during the first 3 weeks, but in the third week, half of the group died. Thiamine deficiency was considered to be the probable cause of death,

since the presence of a thiamine destructive substance in raw starfish had previously been demonstrated. This will be described in detail in the next paper of this series. The diet was therefore supplemented with one microgram of thiamine per gram of diet, which resulted in a marked improvement in the condition of the chicks. Only one more death occurred, so the comparison made in Table 3 is based on the weight of the surviving chicks after 5 weeks of feeding of the supplementary thiamine.

Table 3 - Individual and Average Gains in Liveweight, and Food Consumed Per Gram for the Two Series of Growth Tests with Chickal/

Diet Designation	Gains in Liveweight for Individual Chicks										Average Gain in Liveweight	Food Consumed Per Gram Gain
	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Grams	Grams
Series I												
Pilchard meal	182	225	325	322	203	224	449	408	152	361	285.1	3.32
8% starfish meal ...	538	373	743	383	491	713	565	663	640	*	567.3	2.66
16% " " ...	265	494	374	389	307	341	373	387	372	312	361.4	3.42
2% extracted starfish meal 1st to 3rd wks.	40	36*	38*	22*	55	35	10*	20*	29	41	40.0	5.03
Same plus thiamine 3rd to 8th wks. 2/..	157	-	-	-	247	206	-	-	190	110	182	4.59
3% starfish meal 1st to 3rd wks. ...	12*	45	34	6*	7*	23*	43	22	38	11*	37.0	6.29
Same plus thiamine 3rd to 8th wks. 2/..	-	97	2*	-	-	-	115	177	152	-	135.2	6.05
Series II												
Pilchard meal	970	887	903	873	739	712	696	773	807	967	832.7	3.04
3% starfish meal ...	818	662	837	743	847	859	659	707	757	*	765.4	2.89
6% " " ...	722	906	898	748	735	721	678	805	693	790	769.6	2.90
9% " " ...	604	596	547	707	666	715	613	687	752	-	654.1	2.86

*Dead.

**Dead in 8 days.

1/Duration of tests is 8 weeks unless otherwise indicated.

2/100 micrograms of thiamine added per 100 grams of diet.

The data for individual gains, mean group gains in liveweight, and food consumed per gram gain in weight are shown in Table 3. It is evident that there is a large degree of individual variation in the early stages of growth of the chicks. The data on food required per gram gain in liveweight are about what may be expected for groups showing poor growth. The efficiency of utilization of food is almost invariably below normal for such groups.

In the second series of growth tests, the groups receiving 3 and 6 percent levels of starfish meal showed identical rates of growth, being 8 percent less than that of the group fed pilchard meal. The group receiving 9 percent starfish meal made only 78 percent of the gain in liveweight of the control group. A number of changes were made in the composition of the mash used in this series (Table 2). With the large reduction in maximum level of starfish meal in the diets, the amount of pilchard meal in the control mash was reduced to 3.9 percent of the diet. Soybean oilmeal in all diets was increased to compensate for the fish meal protein that was removed. The adjustments between the mashes were small so that the amount of corn could be kept almost constant in all four mashes. Ground oyster shell was added to the control and to the diets containing 3 and 6 percent starfish meal to balance, approximately, the calcium carbonate in the diet containing 9 percent starfish meal. The range of the calcium:phosphorus ratios was reduced by the addition of 2 percent bonemeal and 3 percent of a distiller's concentrate which was used instead of the dried skim milk as a source of riboflavin.

It seems certain that the poor growth of the control group in Series 1 was due to a deficiency of riboflavin. The better performance of the groups fed 8 and

16 percent starfish meal in the diets may be explained by bacterial synthesis of riboflavin, and possibly other factors, during the early stages of the drying operation in making the meal. The same effect had been noted by Lanham and Nilson (1942) in a study of the possible toxicity of artificially spoiled pilchard meal. In this case also, the diet containing the spoiled meal showed much better results than the diet containing the commercial meal. Further work identified riboflavin as one of the substances that stimulated growth which had been produced during the spoilage of the meal.

In the second series, the control group fed the pilchard meal showed very much greater growth, although they received less than half as much pilchard meal, and 2 percent less crude protein. This was the result of the adequate supply of riboflavin. The relative nutritive values of the pilchard and starfish meal proteins then may be evaluated with the proper prospective.

The sharp decrease in gain in liveweight from 92 to 78.5 percent of that of the control group, which occurred when the starfish meal in the mash was increased from 6 to 9 percent, must be explained on some other basis than as a riboflavin deficiency. The much-depended-upon explanation that the decrease is due to excess calcium or an unbalanced calcium:phosphorus ratio does not appear valid. Five percent of oyster shell was added to the control diet and lesser amounts to the others so as to give all 4 diets a practically identical calcium content: The range was only 2.71 to 2.86 percent calcium, making the range in calcium:phosphorus ratios from 3.2:1 to 3.4:1. The control group and the groups fed diets containing 3 and 6 percent starfish meal made very good growth, with a mean gain in liveweight of 833, 765, and 770 grams, respectively, at the end of 8 weeks.

The extraordinary tolerance of chicks for large amounts of calcium is also evidenced by the surviving chickens fed the high levels of starfish meal in Series 1. With mashes containing 32 percent starfish meal, the diet contained almost 18 percent calcium carbonate with no compensating source of phosphorus (calcium:phosphorus ratio of 11 to 1). Yet these chicks lived and more than doubled their weight in 5 weeks after the thiamine supplementation was started.

There are two other possible explanations for the sharply decreased rate of growth when increased amounts of starfish meal were included in the feed: either the poor quality of the protein or the presence of some other substance carried by the starfish that is detrimental, above certain levels.

All evidence indicates that the starfish protein is very nearly equal to any other marine protein supplement in biological value, when the amount used does not exceed 6 percent. It is suggested that the major factor in the interference with growth of chicks fed levels of starfish meal ranging from 6 to 18 percent, is the presence of thiaminase, the thiamine-destructive enzyme, in starfish meal which has been dried at a low temperature.

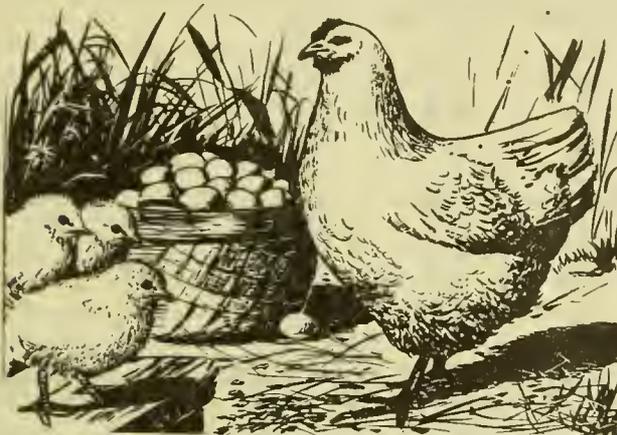
The hypothesis is advanced that the thiaminase content of the diet explains the results of the present test, as well as the similar results noted by Heuser and McGinnis (1946) who fed 6 and 12 percent levels of starfish meal, by Ringrose (1946) who fed 9 and 18 percent levels, and by Whitson and Titus (1946) who fed a 12 percent level of starfish meal. It is notable that a sun-dried meal was used by all these investigators. The one series in which a meal was fed that had been dried in commercial drying equipment (Morse, *et al.*, 1944) resulted in better growth with the 8 percent than with the 4 percent level of starfish meal. This result was obtained with a meal containing only 27.5 percent crude protein

as compared to 30.5 and 34 percent in the sun-dried meal. This would explain why the poor results with sun-dried meals at higher levels are directly due to thiaminase which was destroyed by the heat to which the commercial meal had been subjected during drying.

It is still probable that very high levels of calcium will adversely affect the rate of growth of chicks, but this evidence indicates that as much as 12 percent of starfish meal can be included in the diet and produce good growth if the meal used is entirely free of thiaminase. Thiaminase is not a factor in the use of commercially-dried starfish meals because it is easily destroyed by heat. The probable presence of thiamine should be considered in any starfish meal dried at temperatures of less than 75° C., as it is capable of adversely affecting growth by rendering inactive considerable quantities of dietary thiamine.

STARFISH MEAL IN LAYING MASHES FOR EGG PRODUCTION

The value of starfish meal as a source of protein for growing chicks had been demonstrated, and it was thought advisable to determine its value in egg production.



Commercial laying mash feed contains added sources of lime for egg shell formation. The high calcium content of starfish meal would appear to be a desirable feature in this type of mash, rather than a source of possible trouble.

Since only limited facilities were available for egg production studies, the groups used were smaller than would be necessary to give the desired significance to differences in the results. The only available laying battery consisted of 12 units, permitting only 6 hens

each for a control group and an experimental group.

In the first series of tests, one hen in each group was unproductive or died early in test, so that the final results are based on 5 hens in each group. The hens had been raised from the first series of experimental chicks, the 12 best pullets being chosen from all of the groups. They were fed a stock mash until 6½ months old. At this time all were laying. Hens from all 5 of the original experimental groups were represented, and there was no indication whatever that the retarded early growth on the high-calcium diets had any affect on the future rate of egg production. The composition of the mashes fed during the experimental period is shown in Table 2.

Starfish meal was fed the first group of hens at a 7.5 percent level, being replaced in the control mash by 3.3 percent pilchard meal, 3.7 percent ground oyster shell, and 0.5 percent lard to provide amounts of protein, calcium carbonate, and fat essentially equal in amount to the three chief nutrients of starfish meal.

The results of the first series of laying tests were checked with those of a second series of pullets raised from the chicks of the second series of growth studies. The composition of these mashes is also shown in Table 2. There were

only minor modifications in the formula. Starfish meal was increased to 8.5 percent, with pilchard meal and oyster shell in the control diet increased to 3.67 and 4.83 percent, respectively, to balance the additional starfish meal. Lard was omitted. Soybean oilmeal and corn were increased to compensate for corn gluten meal which was no longer available.

The first laying test favored the mash containing starfish meal in number and gross weight of eggs produced. The mean egg weights were almost identical for the two groups, being 52.1 grams for the group fed pilchard meal and 51.5 for the group fed starfish meal.

Those fed starfish meal laid 435 eggs while the control hens laid 379 eggs; however, one hen of the latter group did not lay for long periods so that on a productivity basis (eggs per day x 100) the groups were nearly identical, being 70.8 for the hens fed starfish meal as compared to 69.7 for the group fed pilchard meal.

Table 4 - Egg Laying Records

Group	Wing Band Numbers	Total Eggs Laid		Total Egg Weight		Average Egg Weight		Productivity - Eggs Per Day x 100	
		Number		Kilograms	Grams			A	B
Series I									
7.5% starfish meal	{	252	89	4.60	51.7				
		226	75	3.84	51.1				
		221	86	4.44	51.6				
		239	104	4.57	43.9				
		242	81	4.79	59.2				
Group Total		435	22.24	-					
Average		87	4.45	51.5			70.8		
3.3% pilchard meal	{	212	75	4.09	54.5				
		250	77	4.20	54.6				
		225	96	4.99	52.0				
		280	74	3.80	51.4				
		299	57	2.73	47.9				
Group Total		379	19.81	-					
Average		75.8	3.96	52.1			69.7		
Series II									
1. Pilchard meal for period A and starfish meal for period B ^{1/}	{	283	39	1.95	50.0				
		289	49	2.56	52.2				
		357	36	2.02	56.0				
		332	39	1.91	50.8				
		346	48	2.32	48.4				
		242	41	2.50	61.0				
Group Total		252	210	13.33	11.51	-	-	66.8	58.8
Average		42.0	35.0	2.22	1.92	53.1	55.0		
2. Starfish meal for period A and pilchard meal for period B	{	317	38	1.96	51.6				
		294	33	1.67	49.2				
		354	14	0.77	55.3				
		329	42	2.07	49.4				
		335	42	1.88	44.9				
		225	42	2.23	55.2				
Group Total		211	204	9.77	10.90	-	-	56.4	57.3
Average		35.2	34.0	1.63	1.82	51.2	54.0		

^{1/}The diets contained 8.5 percent starfish meal and 4.83 percent pilchard meal.

meal over a 4-month period. The individual data for these hens are summarized in Table 4. It is evident that there is a high degree of intra-group variation in both egg size and productivity, so that none of the differences is significant.

The second egg-laying test series was designed to eliminate, to a large extent, the undesirable effect of the individual variation in the hens upon the significance of the results. The design of the test was as follows:

Randomly selected groups, after laying had been well established, were fed mashes containing pilchard and starfish meals, as in Series 1, for 2 months. At the end of this period, the group that had been receiving the mash containing pilchard meal was shifted to that containing starfish meal, and *vice versa*. Egg records were then kept for a second 2-month period. In this way, the individual differences in egg weight and productivity characteristics of the hens in each group were made to apply to each diet for a like period. The individual records of these hens are shown in Table 4, and the results are summarized in Table 5.

Table 5 - Summary of Data for Laying Test Series II

Item	GROUP I		GROUP II	
	Feeding Period		Feeding Period	
	First	Second	First	Second
	Pilchard meal	Starfish meal	Starfish meal	Pilchard meal
Total no. eggs	252	210	211	204
Total egg weight, kg.	2.22	1.92	1.63	1.82
Average egg weight, gm.	53.1	55.0	51.2	54.0
Productivity	68.8	58.8	56.4	57.3

As was expected, there are considerable variations for both individual hens and groups. Those hens originally allotted to the group fed the pilchard meal diet produced both larger eggs and more eggs per hen than the group originally fed the starfish meal diet. After the shift at the end of 2 months, this group still produced more and larger eggs on the starfish meal diet, but the magnitude of the difference was reduced due to the slightly greater nutritive value of the pilchard meal protein. During the second period, there was a decrease for both groups in the number of eggs laid, but an appreciable increase in egg weight, both factors being related to the increased age of the hens. As a combined result of both factors, the total weight of the eggs was almost the same in both periods. Small net differences remained in favor of the group fed the diet containing pilchard meal, both as to number of eggs laid and mean egg weight. The total differences amounted to 35 eggs, and 0.9 gram per egg. The hens receiving starfish meal laid 7.7 percent fewer eggs and the eggs laid were 1.67 percent smaller than those laid by the group fed pilchard meal. The mean productivity was 57.6 for the group fed starfish meal or 7.2 percent less than that of the control group which had a productivity of 62.1.

The data indicate that pilchard meal has a slightly greater stimulating effect than starfish meal on the rate of egg production. The size of eggs laid is almost unaffected by the source of protein in the diet, this factor being apparently hereditary and little influenced by changes in diet. For practical purposes, starfish meal can be rated as a very good source of protein for laying hens, supplying, in addition, all of the calcium needed for shell formation.

CONCLUSIONS

Starfish meal has been fed to newly-hatched chicks at levels varying from 3 to 32 percent of the diet. The starfish meal is only slightly less effective when fed at the lower levels as a source of protein for growth of chicks than is a high-grade pilchard meal. Intermediate levels of starfish had a retarding effect on growth. This effect is thought to be primarily due to the presence of a thiamine-destructive enzyme in meal dried at a low temperature. The excess calcium and unbalanced ratio of calcium to phosphorus may be secondary factors affecting growth, particularly with diets containing more than 10 to 12 percent starfish meal.

Severely retarded growth resulted when chicks were fed diets containing very high levels of starfish meal. Any deaths, however, were due to thiamine deficiency, which also accounted for much of the adverse effect upon growth.

The rat is not a suitable animal for testing growth when fed diets containing much calcium. It has a relatively low tolerance for excess calcium compared with the chick.

Starfish meal compares favorably with pilchard meal as a protein supplement when used in laying mash. It also supplies calcium in place of the ground oyster shell or the limestone which is usually added. The rate of egg production of the group fed starfish meal was 7.2 percent less than for the group fed pilchard meal. This difference, however, is not statistically significant.

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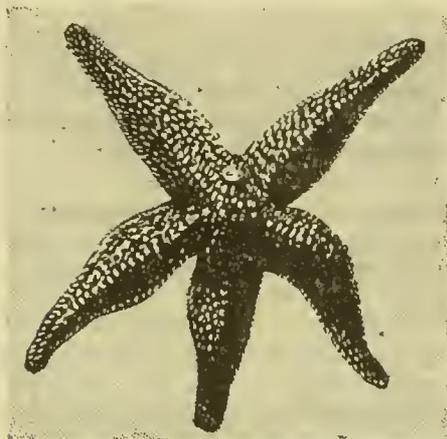


PART IV--THIAMINASE IN STARFISH

By Charles F. Lee*

INTRODUCTION

The first two papers of the series of technological studies on the common starfish (Asterias forbesi) of the Atlantic Coastal waters, discussed its ecological relation to the oyster and review data on the chemical composition of fresh starfish and starfish meal. The third paper discussed data from feeding tests of starfish meal as a protein supplement for growth of rats and chicks and for egg production.



The present paper presents the data of the biological tests from which it was concluded that the starfish contained thiaminase, the thiamine-destructive enzyme.

The presence of thiaminase in starfish has already been mentioned in connection with the protein feeding tests reported in the preceding paper of this series, as a factor affecting growth of certain groups of chicks (Lee, 1948-B). It has also been reported in a review paper on thiaminase (Lee, 1948-A). This review summarized information in the literature on the properties of thiaminase, its distribution in the different

organs and the various species of fish, and also presented a critical discussion of the numerous contradictions regarding the enzyme that are to be found in the literature. No general discussion of thiaminase will be included herein, other than that which seems to be demanded by the present study of thiaminase in starfish.

REVIEW OF LITERATURE

The existence of a substance in fish which actively destroyed thiamine was first proven in 1941, although several years earlier, an investigation of a number of outbreaks of paralysis of foxes had traced the cause to a thiamine deficiency, and implicated fish as the dietary factor responsible for it.

It was eventually concluded that the destructive substance was an enzyme, heat labile at the temperature of boiling water, and separating on dialysis into

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NOTE: Part I of this series, "Starfish Control--Its Economic Necessity and Methods Used," appeared in the January 1948 issue of Commercial Fisheries Review, pp. 1-6. Also available as Sep. No. 193.

Part II, "Chemical Composition," appeared in the February 1948 issue, pp. 11-18. Also available as Sep. No. 196.

Part III, "Value of Starfish Meal--Protein Supplement for Growth of Rats and Chicks and for Egg Production," appeared in the March 1948 issue, pp. 8-19. Also available as Sep. No. 199.

two components of different destructive activity. It is perhaps more accurate to state that the enzyme renders thiamine biologically inactive or unavailable, rather than to refer to its destruction. One process of destruction has been demonstrated to be a hydrolytic splitting of the molecule into its two heterocyclic components, generally referred to as the thiazole and pyrimidine fractions.

It is interesting to note that thiaminase has chiefly been found in what may be broadly called "aquatic animals." All early reports recorded its presence only in fresh-water fish, with the exception of the Atlantic herring. In fish, the greatest concentrations of thiaminase are in the viscera, but it is also widely distributed in the head, skin, and other parts of the fish. There is some controversy as to whether thiaminase is present in the flesh when present elsewhere in the body.

Carp, smelt, and herring are the most important species listed as containing the enzyme, with some 10 or 12 less valuable species; such as, suckers, chubs, burbot, and catfish also containing it. Generally speaking, only a few limited investigations for the determination of the presence of thiaminase have been made of fresh-water species and more especially of the marine species of fish.

Since 1944, thiaminase has been found in a few other strictly marine "animals"; namely, the hard clam, the ocean or black quahog, and the edible mussel. The menhaden has joined the herring as the only true fish in salt water known to contain it.

The occurrence of thiaminase in starfish adds an entirely new phylum of aquatic animals to the list of those which contain the enzyme. That it has not been reported in other echinodermata, or in unrelated types of marine invertebrates, is readily explained by the fact that no assays have been, or seem likely to be, carried out. This type of investigation would serve no practical purpose but might throw light on the present very confusing distribution of thiaminase in nature and on its function in metabolism.

Sautier (1946) included 3 species of starfish from Alaskan waters in his list of fishery products assayed for thiamine by the thiochrome method. These species, Pisaster giganteus, Pisaster ochraceus, and Phycnopia helianthoides, were reported to contain thiamine within the range of 6 to 17 micrograms per 100 grams (5 assays).

The only other reference to the presence of thiamine in starfish, is that of Hutchinson, et al, (1946) who reported 1 milligram per kilogram of starfish meal. They state that, "The low thiamine content of the dried starfish is almost certainly due to post-mortem loss."

Myers (1946), of this laboratory, has found that the thiochrome assay as generally used is of doubtful applicability to the assay of thiamine in fishery products. It is possible that some starfish may contain thiamine and others thiaminase, as similar apparent contradictions have been reported in regard to some species of fish. There are insufficient data to determine the true status of thiaminase, and to correlate its occurrence to such variables as sex, season, maturity, locality, and species. It is suggested that in any fishery product for which a thiamine assay by chemical methods indicates a value of less than 25 micrograms per 100 grams, that the material be tested for the presence of thiaminase by the recovery of added thiamine after incubation under the conditions promulgated for the assay of the enzyme (Sealock, et al, 1943).

THIAMINASE IN STARFISH

The presence of thiaminase in starfish was first detected in the course of a bio-assay intended to determine the thiamine content of raw starfish (Lee, 1948-B). Rats had been maintained on a thiamine test diet (U. S. Pharmacopoeia XI) for a depletion period of 28 days. After this period, rats were assigned to test groups if their weight was less than 100 grams for females, 105 grams for males even though they were still gaining in weight. This unorthodox procedure was necessary in order to assemble a test group within a reasonable time, as the available strain of the rats were quite resistant to thiamine depletion.

Groups of six rats were used on each test level, the animals being evenly distributed as to sex, with no more than one rat from any litter in a group. Rats were housed in individual cages in a room with temperature maintained at $80^{\circ} \pm 2^{\circ}$ F. The assay period was four weeks, with liveweight and food consumption records taken weekly.

The first series was composed of ten groups. All rats received the basal thiamine test diet. Three groups received as supplement $\frac{1}{2}$, 1, and $1\frac{1}{2}$ grams of raw starfish per day, 3 groups were given 20, 50, and 80 micrograms of thiamine per 100 grams of diet, and 3 groups received $\frac{1}{2}$, 1, and $1\frac{1}{2}$ grams of raw oysters as a supplement.

The control rats all continued to gain weight during the test period, averaging 8.1 grams per week. The groups receiving thiamine made much larger gains. Groups receiving 20, 50, and 80 micrograms gained 14.1, 20.8, and 24.0 grams per rat per week, respectively. All rats fed oysters also gained more than the controls.

The rats fed raw starfish, however, did very poorly. Of those receiving $\frac{1}{2}$ gram per day, 1 rat gained in weight, 4 rats lost 8 to 27 grams from their starting weight, although they lived for the 4-week period. One rat was killed on the 23rd day after a loss in weight of 27 grams. When one gram of starfish was fed per day, one rat again gained weight, one lost 39 grams before death on the 20th day of test, and 4 others lost 11 to 29 grams during the 4 weeks. One of these latter rats developed severe polyneuritis, but the symptoms were alleviated and there was a slow gain in weight for 2 weeks after it was given 10 micrograms of thiamine in a single dose. When $1\frac{1}{2}$ grams of starfish was fed daily, the final liveweights were 20 to 34 grams below the starting weights and 4 of the 6 rats developed symptoms of acute polyneuritis. The growth curves for these groups are shown in Figure 1.

In view of the evidence that these rats were suffering from an acute thiamine deficiency, 12 of the rats which had been losing weight were selected from the groups fed starfish and were divided into 2 sub-groups. These were fed the basal diet plus 150 micrograms of thiamine per 100 grams of diet with 1 group getting, in addition, 1 gram per day of raw starfish. In 2 weeks, the former sub-group made an average gain of 66.6 grams, while those getting both thiamine and starfish gained 58 grams.

In the meantime, to determine the possible presence of directly toxic agents in the starfish, 2 groups of 11 rats each were fed the regular stock diet, with 1 group getting a daily supplement of 1 gram of raw starfish per rat per day. Over a 3-week period, the controls averaged 23.7 grams per week, and the starfish group, 22.4 grams per week, an insignificant difference.

Considerable difficulty was experienced in getting the rats to eat the starfish supplements, the material apparently was quite unpalatable. This was particularly true of those rats with unlimited access to the stock diet. A number of the animals receiving depletion diets also refused to eat part or all of the starfish offered. This was notably so for those few rats in these groups which gained weight during the test period.

On the basis of these tests, it was evident that there was present in starfish a substance capable of destroying a limited amount of thiamine. These studies had been started in November 1941, at which time "anti-thiamine," as it was then called, had been reported but very little was known regarding its chemical structure or mode of action. Chemical assay methods had not been developed, so that it was necessary to use the biological assay method for thiamine. By the use of two series of test groups supplemented with thiamine, one with and the other without an additional supplement of raw starfish, it should have been possible to determine the thiamine destroyed per gram of starfish.

This method did not succeed because of the refusal of most of the rats to eat the starfish supplement with sufficient regularity to permit any degree of quantitative comparison. The basal depletion diet was fed alone and with 10, 30, and 60 micrograms of thiamine per 100 grams, with 4 other groups being fed the same diets plus 1 gram per day of raw starfish. During the shortened test period, each of the groups fed starfish gained less weight than the corresponding groups without starfish but the data showed no significant correlations.

A third series of rats was used to determine the practicability of mixing the ground starfish directly into the basal diet, also to ascertain the reported heat lability of thiaminase. Four groups of 6 rats each were used; these were fed the basal thiamine test diet alone; with 10 percent raw starfish; with 10 percent of starfish which had previously been autoclaved for 15 minutes at 15 pounds pressure; and with an amount of starfish oil equal to that which would be introduced by the inclusion of 10 percent starfish in the diet.

Of course, with finely ground starfish mixed into the diet, the only alternative to eating the mixture was fasting. The amount eaten varied depending upon the condition of the rats. It is probable that the starfish produced a depressing effect upon the appetite, in addition to and before the onset of the anorexia resulting from the thiamine deficiency due to the action of the thiaminase.

The control rats in this group gained an average of 5.8 grams per rat per week during the test period; whereas, those fed raw starfish lost 2 to 34 grams, the

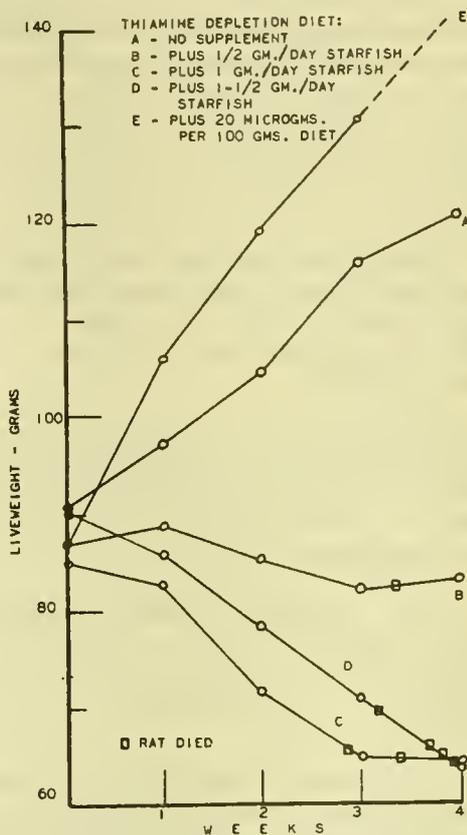


FIGURE 1 - GROWTH CURVES FOR RATS FED THIAMINE BASAL TEST DIET ALONE, AND WITH SEPARATE DAILY SUPPLEMENTS OF RAW GROUND STARFISH AS INDICATED.

average loss being 6.7 grams per week. One rat died after 2 weeks on test while another developed polyneuritis in 3 weeks. The group fed the diet containing cooked starfish had an average loss in weight of 1.5 grams per week, two rats making small gains while the other four lost from 3 to 19 grams. When fed the diet containing starfish oil, 5 of 6 rats gained weight, averaging 7.1 grams per week. The growth curves for these groups are shown in Figure 2.

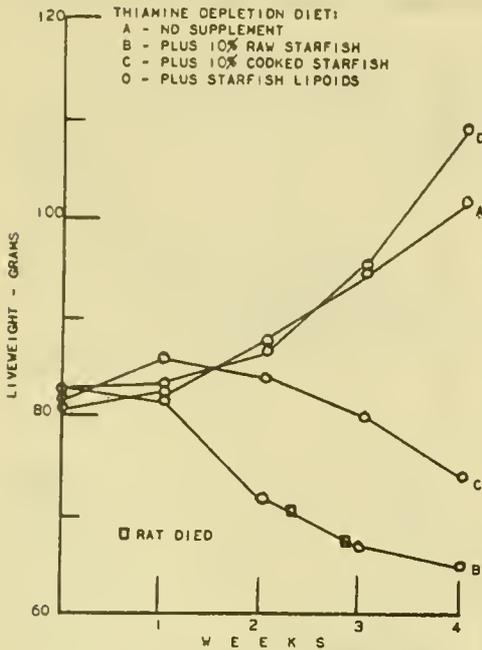


FIGURE 2 - GROWTH CURVES FOR RATS RECEIVING THIAMINE BASAL TEST DIET ALONE AND ADMIXED WITH 10 PERCENT STARFISH, RAW AND AUTOCLAVED, ALSO WITH LIPOIDS FROM STARFISH IN AMOUNT EQUIVALENT TO 10 PERCENT OF STARFISH.

On the basis of this test, it was concluded that starfish oil was in no part responsible for the poor growth which resulted when raw starfish was fed. The destructive principle was not as rapidly and completely destroyed by autoclaving as had been expected, on the basis of reports of heat lability of thiaminase in other materials. This greater heat stability was later confirmed when considerable amounts of thiaminase were found in ground dried starfish meal (Lee, 1948-B).

Relatively heat stable forms of thiaminase have more recently been reported in Indian oil seeds (Bhagvat and Devi, 1944) and in a fern, *Pteris aquilina* (Weswig, et al, 1946). It now seems probable that "thiaminase" is not a single enzyme, but rather that there are a number of thiamine-destructive principles differing in heat stability, properties on dialysis, and presumably in chemical structure.

Another attempt at a quantitative determination of the thiaminase in starfish was made with a fourth series of rats fed on the thiamine test diet plus 10 percent ground raw starfish, and a group of thiamine supplements. From 10 to 100 micrograms of thiamine per 100 grams were fed in the diet containing starfish, with a control group being fed the basal diet plus 25 micrograms of thiamine per 100 grams. A new lot of starfish had been obtained for this test to lessen the chance of loss of thiaminase which may have occurred in handling or storage of the first lot. The test indicated, however, that the new starfish sample contained less thiaminase than the previous sample. The group fed the basal diet plus 10 percent starfish of the fourth series showed a loss of weight of 2.4 grams per rat per week, only 36 percent of the weight loss of the group in the preceding series fed an identical diet except for the lot of starfish.

The growth curves of this test series are shown in Figure 3. About the best estimate of the amount of thiamine destroyed is 4 micrograms per gram of starfish. The fact that the thiaminase content was lower than was expected led to a selection of supplement levels with too great a spread for accurate evaluation in this low range. The test was not repeated, however, as the procedure was not satisfactory when raw starfish was fed, and results did not justify use of further time and additional rats for this purpose.

THIAMINASE IN STARFISH MEAL

It has already been noted that thiaminase remaining in starfish meal was a factor adversely affecting the growth of chicks fed a mash formula used to determine the value of the meal as a protein supplement. These chicks had been fed on high levels of starfish meal; namely, 32 percent regular meal and 29.5 percent extracted meal. These levels were fed primarily to determine the tolerance of the chick for excess calcium, and an unbalanced calcium:phosphorus ratio. Thiamine in the mash was supplied by bran, middlings, and other grain products, and was adequate in the diets containing pilchard meal and the lower levels of starfish meal.

There were no deaths in these groups until the 13th day of test, but 11 of 21 chicks died during the next 8 days. It was thought possible that this heavy mortality after 2 weeks might be due to a thiamine deficiency resulting from the presence of thiaminase which had not been destroyed by the low drying temperatures. After 3 weeks, therefore, 100 micrograms of thiamine per 100 grams of mash was added to both diets containing the high levels of starfish. The surviving chicks showed much improvement in condition and fairly good growth response during 5 weeks they were fed on the diets supplemented with thiamine as shown in Figure 4.

The slightly better growth of the group fed the extracted meal was probably due to the extraction or destruction of some thiaminase during the prolonged extraction with hot acetone. The thiamine supplement was therefore doubled for the group fed the regular meal after 2 weeks on the 100-microgram level, and a further response in growth was obtained (see Figure 4).

Growth, even when the diet was supplemented with 200 micrograms of thiamine per 100 grams of mash, did not approach normal for the age of the chicks. There is no proof that even this relatively large amount of thiamine supplied an optimum level. Feeding tests of the same type conducted by other investigators have shown that the addition, for example, of 200 micrograms of thiamine to a mixture containing thiaminase may result in the loss of 90 percent, which is equal to 180 micrograms of thiamine. If much larger amounts, perhaps 1,000 micrograms, were added, larger actual quantities, but a smaller proportion of the total, for example, 700 micrograms, may be destroyed by the same amount of thiaminase.

On the other hand, the excess calcium carbonate, amounting to almost 18 percent of the diet, was undoubtedly also a factor tending to retard growth but the

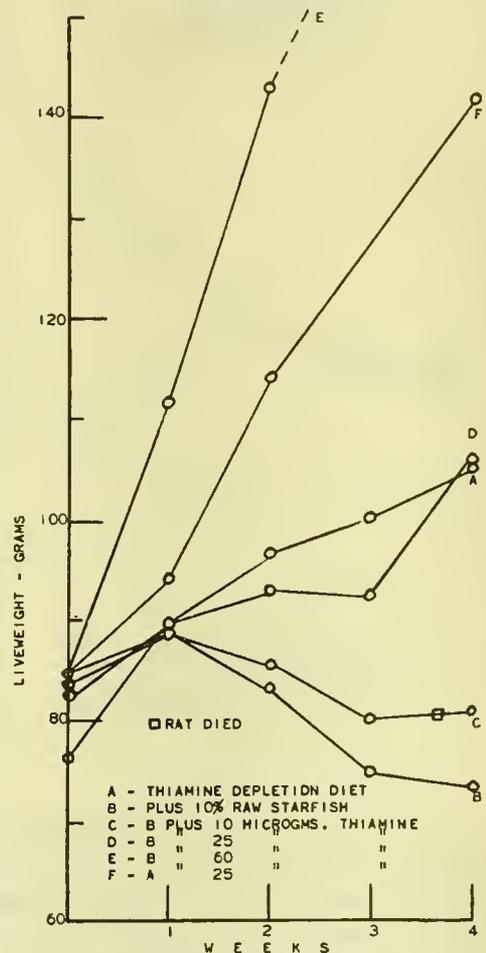


FIGURE 3 - GROWTH CURVES FOR RATS RECEIVING THIAMINE BASAL TEST DIET ALONE AND ADMIXED WITH 10 PERCENT STARFISH, BOTH WITH AND WITHOUT SUPPLEMENTS OF 10, 25, AND 60 MICROGRAMS THIAMINE PER 100 GRAMS DIET. ALSO THE GROWTH CURVE OF THE GROUP FED THE BASAL TEST DIET WITHOUT STARFISH BUT PLUS 25 MICROGRAMS OF THIAMINE PER 100 GRAMS.

separate evaluation of the two effects is impossible from the present data. It is evident, however, that the mortality was primarily the result of a thiamine deficiency due to the thiaminase content of the starfish meal rather than to excess calcium.

CONCLUSIONS

Raw starfish, *Asterias forbesi*, contains a thiamine-destructive enzyme, commonly called "thiaminase." This was demonstrated by the development of polyneuritis in rats fed one or more grams of raw starfish per day. Recovery and normal growth resulted from the addition of an adequate level of thiamine to the diet.

It was difficult to obtain a quantitative estimate of the amount of thiamine destroyed because of the refusal of rats to eat the raw starfish with any regularity. The best estimate is that one gram of starfish inactivates four micrograms of thiamine. There are indications that other samples of starfish may contain greater amounts of thiaminase.

The thiaminase in starfish is sufficiently stable to remain in starfish meal that has been sun-dried or dried at low temperatures. There are no data to show the amount of thiaminase lost under different conditions of drying.

Thiaminase in starfish meal is primarily responsible for mortality of chicks fed diets containing high levels of this product. This factor and the high calcium content of the starfish meal are responsible for the poor growth obtained. Evidence from other sources indicates that thiaminase would be destroyed at the temperatures commonly used in the dryers in the commercial production of protein meals from fishery products. Any small amount of thiaminase remaining would not give much response since usually less than 10 percent of mixed animal protein supplements are included in commercial mash formulas.

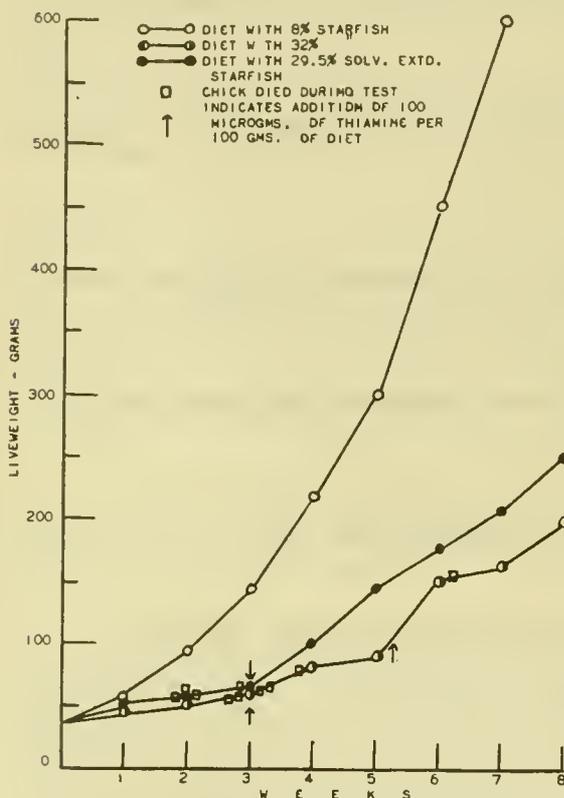


FIGURE 4 - GROWTH CURVES FOR NEWLY HATCHED CHICKS, FED EQUI-PROTEIN MASHES CONTAINING 8 PERCENT STARFISH MEAL, SHOWING NORMAL GROWTH. ALSO CURVES FOR CHICKS FED 32 PERCENT STARFISH MEAL DRIED AT A LOW TEMPERATURE, AND 29.5 PERCENT OF SAME MEAL WITH LIPIDS EXTRACTED BY ACETONE. THE LATTER TWO DIETS WERE SUPPLEMENTED BY 100 MICROGRAMS THIAMINE PER 100 GRAMS OF DIET AFTER 3 WEEKS, AND THE 32 PERCENT STARFISH MEAL MASH HAD THE SUPPLEMENT OF THIAMINE INCREASED TO 200 MICROGRAMS OF THIAMINE AFTER 37 DAYS, AS INDICATED BY ARROWS.

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PART V--STARFISH AS FERTILIZER

By Charles F. Lee*

INTRODUCTION

The four previous papers of this series of technological studies of the starfish (Asterias forbesi) have reported data in the literature, as well as those obtained in these investigations on the chemical composition of starfish, use of starfish meal as a protein feedstuff, and on the presence of thiaminase in raw starfish and starfish meal. The present paper gives the results of an investigation on a small local scale directed towards the better utilization of starfish as fertilizer.

Most of the reports on the utilization of starfish prior to 1942 have been concerned with its value as fertilizer (Kole, 1919 and Vachon, 1920). Since 1942, the dried starfish meal has been found to have sufficient value as a protein supplement for poultry feeds so that any meal which could be produced could be used for this purpose. The only recent reports of its use as fertilizer have been limited to the use of whole raw starfish. The starfish landed at Providence during the period when the State of Rhode Island was paying bounty on the pests were disposed of in this way (Gibbs, 1941 and 1946). There have been other unpublished reports of similar use of small quantities on private gardens and small farms.

In general, reports of the use of starfish for fertilizer have been quite favorable. So far as is known, however, none of the trials have been adequately planned, with control and competitive plots, to permit comparison of starfish with balanced commercial fertilizers.

Starfish, on the basis of chemical analysis, is a source of organically bound nitrogen, containing negligible amounts of phosphorus and potassium, the latter two elements being of primary importance in balanced fertilizers. The ash content, largely calcium carbonate, may amount to 60 percent by weight of the meal and dilutes the nitrogen to a fairly low value, less than 5 percent on a dry matter basis. As a fertilizer, this calcium carbonate is a desirable addition for acid or "heavy" soils. Starfish were not being used in any way and since they have some fertilizer value, an investigation was made to determine a practical way to use raw starfish as fertilizer. Most starfish are taken in the spring and summer, and piles of raw, untreated starfish decompose rapidly at the temperatures prevailing during these seasons. The odors thus developed would condemn handling

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in this manner, inasmuch as most of the starfish are landed in sizable cities, such as New Haven and Milford, Conn., and Providence, R. I.

Because of the rapidity with which starfish decompose, it is the general practice for those taken in control operations to be shoveled overboard before reaching port. The suggestion was made that if these starfish could be treated so as to delay decomposition, sufficient quantities might be accumulated to justify hauling the material some distance to farms where it could be used as fertilizer. In this way, small and scattered amounts of starfish could be utilized when and where landed, largely eliminating adverse factors of cost of transportation and irregularity of supply which practically prohibit the use of starfish as raw material in fish meal drying plants.

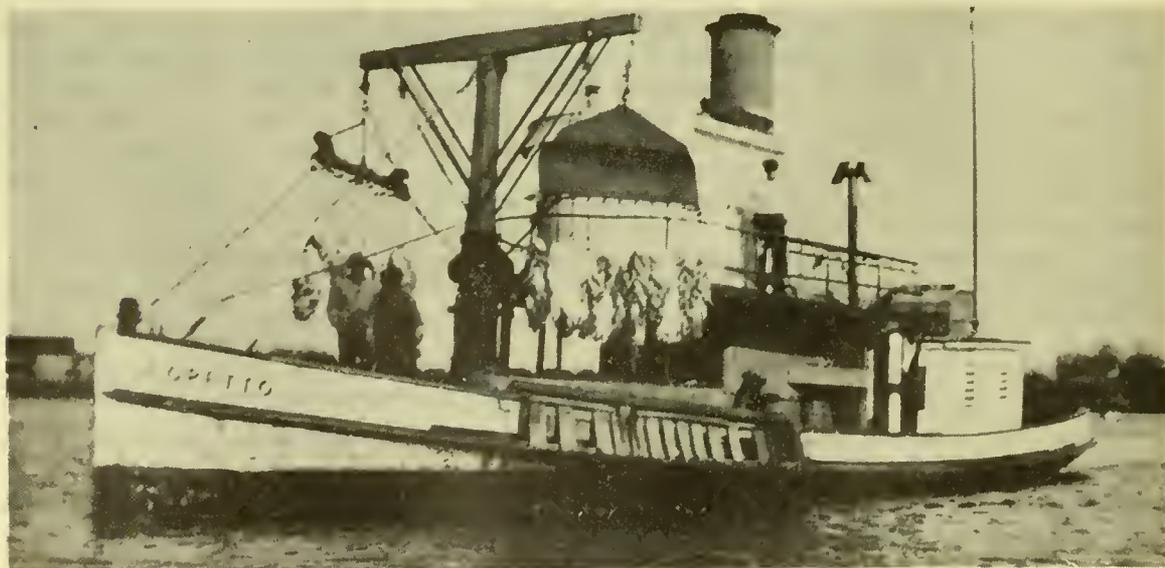
EXPERIMENTAL

In the early days of the menhaden oil industry, sulfuric acid was often used to delay decomposition of the presscake until enough had accumulated to operate the driers. For this purpose, 3 to 5 percent sulfuric acid by weight gave the desired result. The suggestion was made that a similar small amount of acid might be equally effective on starfish.

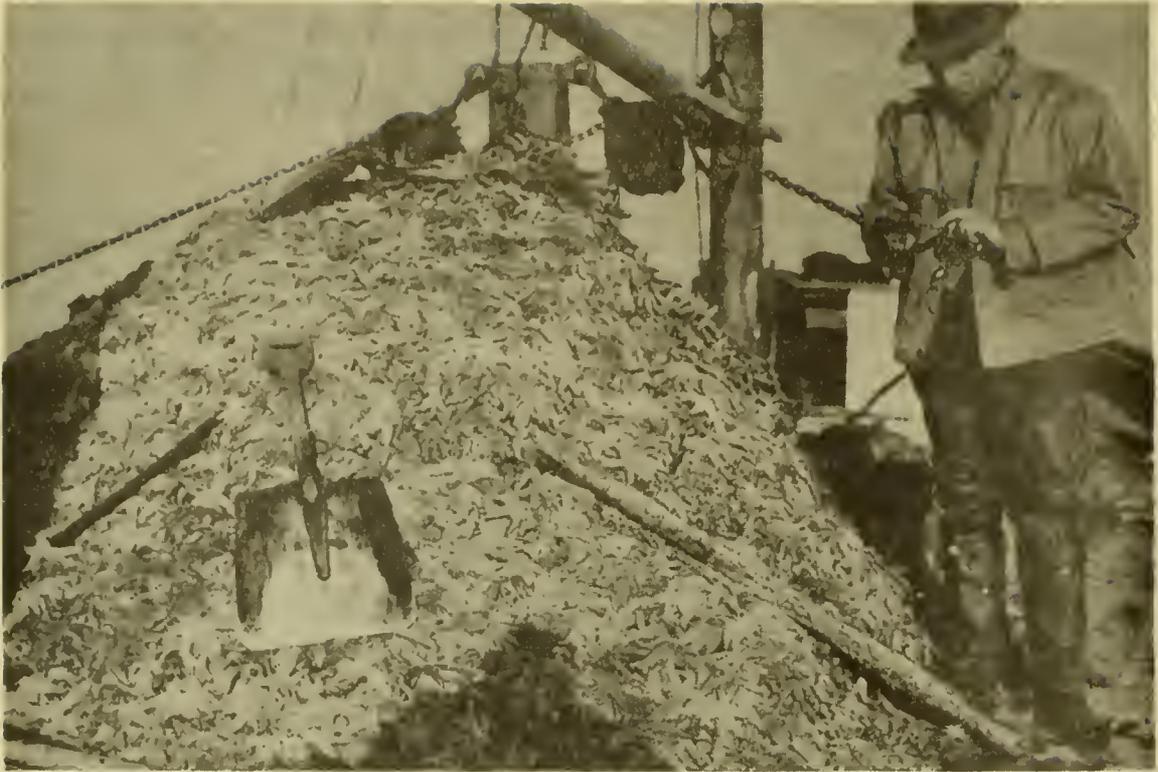
Therefore, a field trial was planned, using freshly caught starfish handled in a manner similar to that necessary in any practical adaptation of the process. Various quantities and concentrations of sulfuric acid were used to determine the optimum conditions for the desired effect.

Preliminary tests in the laboratory had shown that the starfish could withstand fairly concentrated acid without rapid structural breakdown. For greater safety in handling, the first tests were carried out with dilute acid.

Concentrated sulfuric acid was siphoned into a quart measure and added to an amount of water in a stoneware crock, which would give the desired dilution. This



TYPICAL STARFISH BOAT, OPERATING IN LONG ISLAND SOUND, EQUIPPED WITH MOPS



STARFISH CAUGHT IN FOUR HOURS BY A CRAB DREDGE BOAT (CRABBING) IN CHESAPEAKE BAY

technical grade acid, 56 Baume acid, cost at that time about 3 cents a pound in 5-gallon carboy lots. Great care is needed in the mixing operation. The concentrated acid is rapidly, and the dilute acid more slowly, corrosive to both skin and clothing, and the dilute acid rapidly attacks any metallic equipment unless lead lined. Rubber gloves and rubber equipment are desirable as protective clothing, and a stoneware crock is the best mixing container usually available. In the use of stoneware, care is also necessary to prevent cracking from the heat generated by too rapid addition of acid to water. The above properties should be considered because of the danger which would be involved if the acid were to be handled by oystermen or farmers without special equipment and knowledge of the precautions necessary for safety.

The starfish used in the present investigation had been picked from mops by the starfishing crews of a New Haven, Conn., company, and the acid treatment was carried out in the grounds of the plant. Two lots were collected, on October 28 and 30. Approximately 200 pounds of starfish in the first lot were divided into three piles. No facilities were available for weighing the material so all quantities were estimated. The 3 batches of starfish were then treated with 3 dilutions of acid, 1 to 9, 4 and 2, parts of water by volume, respectively. An equal quantity, 6 quarts, of each dilution was used, and this amount was sprinkled slowly over the piles during the course of about one-half hour. The starfish were turned and mixed with a fork several times during the process.

There was a rapid initial action of the acid with exposed carbonate in the ambulacral spines, etc., but this soon slowed down, and a considerable amount of

the acid thereafter ran off to the ground and was lost. The calcareous, exoskeleton makes the starfish very non-absorbent and difficult to treat by ad-mixture of any liquid in this manner. The fact that the calcium sulfate formed by the acid action is only slightly soluble in either acid or water further tended to hinder the reaction going rapidly to equilibrium.

In treating the second lot of starfish, a single concentration of strong acid, 1 part of acid to 1 of water by volume, was used in an effort to lessen the loss by run off, and the volume of acid was varied. The lot was divided into 4 batches which were treated with 2, 3, 4, and 7 quarts, respectively, of the 1 to 1 acid in the same manner as the first lot. The weights of the individual batches were estimated to be about the same as for lot 1, or approximately 70 to 80 pounds each.

After treatment, the starfish were allowed to remain in a pile for a time in order to allow absorption of as much of the acid as possible. They were then raked out into a thin layer on the ground to dry. Threatening rain on October 29 made it necessary to take the first 3 lots into the shelter of a shed. October 30, the weather cleared and all 7 lots were spread outside to dry. Unfortunately, during the night, the whole area was flooded by an exceptionally high tide, so that all piles were under water the morning of October 31. Some starfish floated off but the bulk of each lot was forked out of the water and moved to high ground. The next day, rain again made it necessary to move the starfish inside so that on the following day the material was packed into barrels and shipped to College Park.

RESULTS

At the time of shipping, decomposition had already begun in the batches treated with 6 quarts of 1 to 9 acid and 2 quarts of 1 to 1 acid and only small amounts of these batches were shipped (batches 1 and 4, Table 1). Some spoilage was also

Table 1 - Results of Treating Starfish with Sulphuric Acid

Batch number	Concentrated acid used	Approximate part of acid used to 100 parts starfish	Nitrogen, dry basis	Calcium carbonate, dry basis	Approximate efficiency of acid action
	Pounds		Percent	Percent	Percent
Lot 1:					
1	2.30	3.5	4.72	46.4	96
2	4.60	6.5	4.23	38.8	77
3	7.95	11.0	4.47	32.4	59
Lot 2:					
4	3.85	5.5	4.79	43.0	73
5	5.75	8.5	4.70	35.8	71
6	7.65	11.0	4.52	32.1	62
7	13.40	19.0	4.43	22.9	47

evident in the batches which had been treated with 6 quarts of 1 to 4 acid and 3 quarts of 1 to 1 acid (batches 2 and 5, Table 1). The condition of the other 3 batches indicated fair preservation, evident by the comparative stiffness of the starfish, which normally mat together quite cohesively. This matting makes it difficult to sun-dry fresh starfish, and consequently, the acid treated samples would dry relatively easily and rapidly, given suitable weather.

On arrival November 5, at College Park, 6 and 8 days after treatment, inspection confirmed previous conclusions as to the effectiveness of the various treatments. Only the batches treated with 6 quarts of 1 to 2 acid, and 4 and 7 quarts of the 1 to 1 acid solutions were fairly well preserved (batches 3, 6, and 7, Table 1).

In Table 1, the amount of concentrated acid used in each treatment and the approximate proportion of acid to starfish (column 3) has been calculated. Samples of each batch were ground and analyzed for nitrogen and carbonate. On the basis of previous analytical data, it was estimated that the initial nitrogen content was 4.90 percent and the calcium content was 58 percent of the dry matter.

These data (Table 1 and Figure 1) indicate that the nitrogen content is not greatly affected by the acid treatment, but that the variation which does occur is opposite to that which would be expected. In general, the lots which received the most acid have the lower nitrogen content. Since the condition of the samples did not indicate that this result could have been due to post-treatment decomposition of protein, which should have led to the exactly opposite effect, it would appear that the nitrogen was lost at the time of acid treatment, presumably by hydrolysis of part of the starfish protein to a soluble form which was then leached out.

The calcium carbonate remaining is a measure of the extent of the action of acid with this component. A greater proportion of acid reacted with carbonate at the lowest levels of acid used. As the amount of acid was increased, the amount of unchanged calcium carbonate decreased as shown in Figure 1. The proportion of acid which reacted decreased also, until when the most acid was used only about one-half of the acid was found to have reacted with carbonate.

A portion of the excess acid must have reacted with or have been absorbed by the protein, as some preservative effect was obtained. However, most of the acid which did not form sulfate was most probably lost on the ground as run off. Even when the acid used amounted to nearly one-fifth of the weight of the wet starfish, it did not neutralize much more than 60 percent of the calcium. The excess alkaline ash must soon have restored the mixture to a nearly neutral reaction; whereas, with the menhaden type of fish meal, small amounts of acid are sufficient to maintain an acid reaction.

CONCLUSIONS

Raw starfish, on the basis of chemical analysis, is rated as a fair source of nitrogen for fertilizer and as a poor or unimportant source of phosphorus and potassium. The calcium carbonate it contains would be beneficial when used on acid or heavy clay soils.

Treatment with sulfuric acid is not a very effective means of preventing decomposition of raw starfish to facilitate its use as fertilizer because of the large quantities of acid required. To delay decomposition, concentrated acid amounting to 15 percent of the weight of the raw starfish is required.

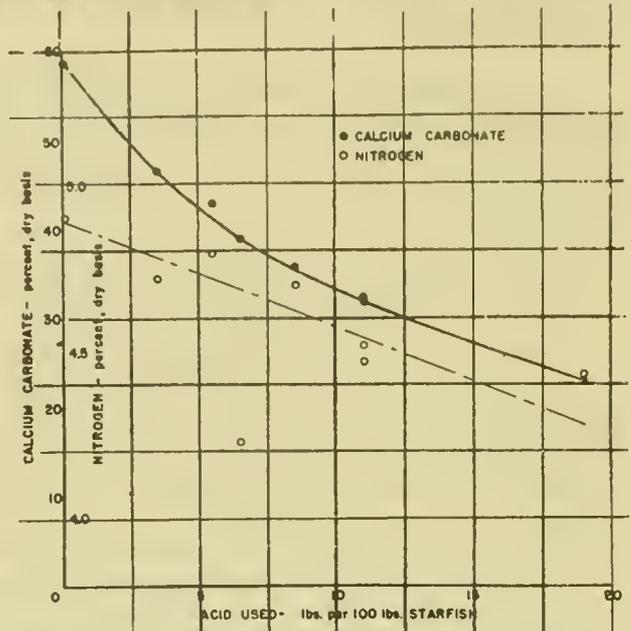


FIGURE 1 - RELATIONSHIP OF THE CALCIUM CARBONATE AND NITROGEN CONTENTS OF RAW STARFISH (DRY BASIS) TO THE AMOUNT OF CONCENTRATED SULFURIC ACID USED IN THEIR TREATMENT FOR FERTILIZER.

The cost of acid, estimated to be \$35.00 per ton of dry meal, is excessive considering the relatively low value of the starfish as fertilizer. Acid treatment further decreases the fertilizer value in two ways: by loss of nitrogen, presumably by leaching of a soluble protein hydrolysate; and by replacement of part of the alkaline acting calcium carbonate by the neutral, relatively insoluble calcium sulfate.

Acid treatment of starfish involves danger to person and property in the hands of inexperienced persons lacking special equipment and protective clothing.

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PART VI - ECONOMIC CONSIDERATIONS IN THE UTILIZATION OF STARFISH

By Charles F. Lee*

INTRODUCTION

Previous papers have discussed the relation of the common starfish (Asterias forbesi) to the oyster industry and the phases of starfish utilization which have been investigated by the U.S. Fish and Wildlife Service. The economic considerations involved in any practical utilization of starfish have been mentioned only briefly before. It is the object of this concluding section to investigate this important phase of the general problem of the utilization of starfish in the New England area.

SUMMARY OF POTENTIAL USES FOR STARFISH

Briefly, the investigations of the Fish and Wildlife Service have been confirmed by several other investigators with respect to the value of starfish meal as a feedstuff. It was found to be a valuable protein supplement in amounts up to 6 percent by weight of growing mash for chicks. In addition, starfish meal satisfactorily supplied both protein and lime in laying mash at a level of 8 percent. Raw starfish as well as meal dried at low temperatures were found to contain thiaminase, the thiamine-destructive enzyme. This added a new phylum of marine organisms to the list of those with members containing thiaminase. Raw starfish used as fertilizer supply about 1.3 percent available nitrogen and 3.5 percent of acid soluble calcium. Treatment with sulfuric acid does not, however, solve any of the problems involved in handling and storing large quantities of raw starfish.

The proximate analysis of starfish does not indicate any other way in which starfish might be used. Starfish oil must be solvent extracted as it averages about 2 percent and rarely exceeds 3 percent of the freshly caught material. The oil has been found to contain a complex mixture of virtually inseparable sterols (see Part II). So far as is known, none of these sterols shows promise as intermediates in the fields of vitamin or hormone chemistry. Only the existence of a high-priced byproduct would justify the costly solvent extraction of the small amount of oil available. Thorough investigation of the protein of starfish offers some promise of discovery of a product of high value. The protein is readily broken down and might prove to be a source of certain amino acids which have recently been in considerable demand for clinical studies and nutrition research.

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Part IV, "Thiaminase in Starfish," appeared in the May 1948 issue, pp. 12-19. Also available as Sep. No. 204.

Part V, "Starfish as Fertilizer," appeared in the June 1948 issue, pp. 11-16. Also available as Sep. No. 206.

ECONOMIC FACTORS RELATING TO USE OF STARFISH FOR PROTEIN MEALS

Special handling of starfish in any quantity, large or small, would be justified if the material were to be used in preparation of amino acids or vitamin and hormone intermediates. However, at present, the only proven value of starfish is as a source of protein in poultry feed or in fertilizer. For these purposes, it is in direct competition with the other protein byproducts. Some of these are crab scrap meal, shrimp and lobster bran, and the "white fish" meal produced from New England groundfish fillet scrap. In fact, since starfish meal is merely a potential source of protein dependent on economic factors, other potential sources might be used under certain circumstances. Of these might be mentioned the enormous quantities of trash fish discarded by the North Atlantic trawl fisheries, as well as the smaller, but sizable, quantity



STARFISHING VESSEL

of trash fish taken, but not utilized, by the shrimp trawlers in the South Atlantic and Gulf.

For this reason, the creation of an industry based on the use of starfish as a raw material for the production of protein meals is dependent upon a number of factors, each directly affecting its economic feasibility. To be considered are:

1. The amount of starfish available from present control efforts of the oyster industry, and costs thereof.
2. The regularity of supply from month to month and over a period of years.
3. The possible quantity of starfish to be obtained from a separate fishery and costs of such operations.
4. The cost of production, transportation and marketing of starfish meal.

It is virtually impossible to obtain data on the catch of starfish, cost of control operations, fluctuations in the number of starfish and other pertinent information (Galtsoff and Loosanoff, 1939 and Burkenroad, 1946). Starfish are regarded by oystermen as a necessary evil to be kept at the lowest level consonant with a reasonable expenditure of money and effort. Operating costs of vessels used for starfish control vary widely with the type and size of vessel used and the method of control. In 1947, these were estimated to be \$35 to \$50 per day at a minimum, while costs may exceed \$150 per day per vessel when the large oyster dredge boats are transferred to cleaning grounds of starfish.

The amount of starfish taken by these control efforts is even harder to estimate. Generally, the starfish have not been brought to shore so that a quantitative estimate is not possible. The starfish are landed on deck only when the mops are hand-picked or during the uncommon occasions when starfish are dredged. Catch estimates of starfish taken by the mops which are dipped in hot water are, at best, rough estimates. The material taken by dredge may consist of more crabs, conchs, oysters, shells, and rocks, than of starfish. If the amount of starfish exterminated

is not known, at least it is generally agreed that the quantities of starfish encountered show large variations from year to year and even from month to month (Sweet, 1946). At certain times, every available craft is working at starfish control, while during similar periods in other years so few starfish may be found that the only operations necessary are periodic surveys to detect any sudden increase in population which can then be checked before serious damage is done.

Unpublished work of Loosanoff suggests that the abundance of starfish for a given season can be predicted with some degree of accuracy from a study of larval forms in plankton samples taken in the preceding months. However, very little is known of the causative factors in the fluctuation in abundance of starfish. The opinion has been prevalent both among growers of seed oysters and the State agencies of Rhode Island and Massachusetts that the starfish population can be materially reduced for some years by intensive control efforts during periods of heavy infestations. This theory has been the basis for the limited appropriations which have been made several times in recent years paying a bounty on starfish caught, (Barnes, 1946 and Gibbs, 1941 and 1946).



CLEANING MOPS ON STARFISHING
VESSEL

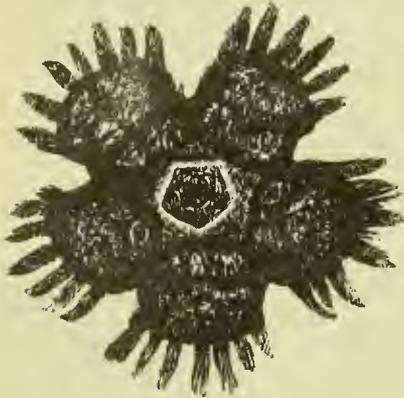
On the other hand, the trend in recent years has been for biologists to attribute more and more weight to the effects of ecological factors on the size of populations. Many of these factors are still unidentified. The effect of human factors, such as, hunting, sport fishing, extensive commercial fishing, trapping, and even bounty payments for predators are often believed to be secondary in importance in their effect on future abundance.

Some of the species, the abundance of which is held to be greatly affected by these ecological factors, are certain of the game birds and smaller game animals, fresh-water game fish, and marine species of fish and shellfish, such as, the blue crab, haddock, mackerel, menhaden, and pilchard. This does not imply that too heavy hunting or fishing cannot significantly reduce a population, but in normal years, it has been estimated that, for some of the marine species with a short life cycle, a capture of as much as 80 percent of the population will not materially affect future abundance. Conversely, disease, drought, abnormal rainfall, and similar uncontrollable conditions may dramatically reduce a population, for many years in some cases. The well-known mystery of the disappearance of smelt in the Great Lakes is an illustration.

It is not too surprising, therefore, that Burkenroad (1946) found evidence of a large annual variation in his extensive, though hardly quantitative, survey of starfish abundance. In the course of fluctuations of a seemingly cyclic character, he estimated a decrease in the population of the order of one-twentieth of

that found at the maximum. The nature of the information on which these conclusions were based does not permit quantitative comparison of the population density at the several maxima.

It was suggested, though also not subject to proof, that the fluctuations in starfish abundance coincided throughout the whole New England area. Since control efforts have been carried on by the oystermen throughout the period studied, it is of course impossible to separate their influence from that of natural factors. This is emphasized by the fact that most of the information comes from sources directly influenced by the reports of oystermen on starfish abundance, namely, trade journals and newspapers.



STARFISH ABOUT 1 MONTH OLD

Actually, for present purposes, it does not matter whether the fluctuation is man-made or from natural causes. The critical fact is that enormous variations in abundance do occur. One company encountered a range from 5 to 650 tons per year in its estimated catch of starfish. The supply of raw material from a fishery of this type does not permit the economical operation of a meal drying plant.

A rough estimate of the cost of starfish taken by the seed oyster companies may be made based on average costs of \$50 per day to operate a vessel taking 8 bushels of starfish weighing approximately 500 pounds. A ton of raw starfish would cost \$200, which is equivalent to a cost of \$1,000 for raw material to produce a ton of meal. This figure probably would be at least doubled if the starfish were hand-picked. The drying plant, on the other hand, could not pay more than \$3 to \$4 per ton for raw material.

The establishment of a separate fishery for starfish comes somewhat nearer to the border of economic feasibility. A bounty was paid on starfish landed in Massachusetts from 1932 to 1936 (Barnes, 1946) and in Rhode Island in 1941 (Gibbs, 1941 and 1946). There was also one commercial plant at Mobjack Bay, Va., which made starfish meal for a short period in 1935-36 when starfish invaded the lower Chesapeake Bay. From these sources, an estimate of the cost of a separate fishery for starfish may be made. The starfish dredged from Chesapeake Bay were estimated by Burkenroad to have cost the Virginia meal plant from \$2.50 to \$4.00 per ton. Bounty payments have ranged from \$10.00 to \$15.00 per ton, the price being increased as the abundance of starfish decreased. Bounty payments were limited to starfish taken from small skiffs with hand dredges. With an organized fishery using much larger, powered fishing craft, costs could undoubtedly be reduced below these figures. However, with the high operating costs of the postwar period, it would be difficult even in periods of maximum abundance to land starfish at a drying plant for as little as \$5.00 per ton. Over a period of years, the previously discussed uncertainty of supply would make the average cost of raw starfish several times this figure, or a far greater cost per ton of dry meal than its retail value.

MEAL PRODUCTION COSTS

A suggestion of possible merit would be the construction of a meal plant designed for processing starfish during periods of maximum abundance with the use

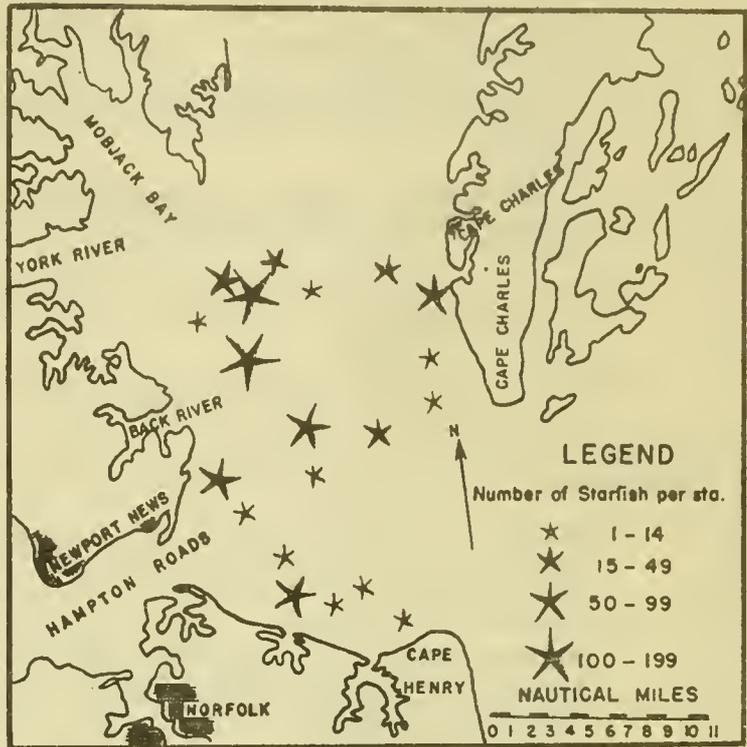
of other raw materials, such as trash fish during periods when starfish are relatively scarce. The existence of such a standby source of unused raw material would have to be assured. The relatively small size of the Connecticut trawl and trap fishery up to 1947 has not offered the assurance of a reliable supply of trash fish.

With raw material costs inevitably high, transportation costs would of necessity have to be kept at a minimum. The drying plant would have to be located at the point of maximum starfish concentration. A floating dehydration plant would solve the problem of accessibility to a shifting and uncertain source of raw material. To operate efficiently, this type of plant would need a small fleet of "buy" boats to collect the starfish. Since operations of this type have not been carried out on the East Coast, cost estimates are difficult to make. It is certain that

costs would be very high unless a supply of raw material of many times the quantity of starfish now available in 1947 were definitely assured.

The cost of drying, grinding, packing, and selling of starfish meal would be equal to or greater than similar costs of other byproduct meal. Personal observation, as well as the limited experiences of the Rhode Island Oyster Company in producing a trial lot of starfish meal, suggest that the tendency of raw starfish to mat together will lead to difficulties in maintaining an even feed to the driers. Special handling would be required to eliminate this difficulty, and grinding the dry meal might also present difficulties. The starfish skin is both tough and abrasive, and has a tendency to flake into sheets rather than to break into a uniform particle size. Reduction of moisture content below 3 percent would facilitate grinding but would add considerably to drying costs.

The most efficient type of drier operating continually at optimum capacity would add \$19.00 to \$20.00 to the cost of a ton of starfish meal. Total production costs were estimated by Burkenroad to total about \$42.00 per ton for a steam dried meal. This value is, however, based on a regular year around supply of raw material to yield an annual production of 5,000 tons. This would mean 25,000 tons (50 million pounds) of raw material would be required and as indicated above there seems to be no possibility that the supply of starfish could regularly meet more than a small fraction of this total demand for raw material.



DISTRIBUTION OF STARFISH IN CHESAPEAKE BAY IN MARCH 1937

CONCLUSIONS

1. The production of starfish meal is not practicable for the following reasons:

- A. Control methods practiced by the oyster industry do not offer a reliable source of raw material.
- B. A separate fishery for starfish could not operate at present to yield raw material at a cost consistent with its value as a feedstuff or fertilizer.
- C. Extreme annual fluctuations in the abundance of starfish creates a very poor source of supply of raw material for a meal drying industry, regardless of the cost of raw material.
- D. There are no byproducts, such as oil, which might carry part of the production costs. The costs would be as high or higher than for any other byproduct meal.
- E. Starfish meal has a low nitrogen content and high ash content and therefore is a relatively low priced product.

2. Control operations now practiced by individual oyster companies appear to be the best means for combating the menace of starfish to the oyster industry.

- A. Reduction of starfish population by bounty payment is only temporary. It appears probable that abundance will normally decline from maximum through natural causes within one or two years.
- B. Further biological research is needed to prove the existence of an abundance cycle, and to study larval forms of plankton samples in order to predict the abundance of starfish in the immediate future. Reliable information of this nature should enable more efficient and intelligent planning and utilization of present control equipment by the oyster companies.

3. Future technological research on starfish should be directed to the development of high-priced preparations from starfish.

- A. The utilization of starfish for feed or fertilizer has been sufficiently explored to show that it is theoretically possible but not economically feasible.
- B. At the present time, it would appear that development of methods for the separation of the amino acids of the protein of starfish might produce products of sufficiently high price to encourage the establishment of a separate fishery.

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